University of Alberta

The prevalence and ecological significance of root grafting in lodgepole pine

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Forest Biology and Management

Department of Renewable Resources

Edmonton, Alberta

Spring 2006

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Your file Votre référence
ISBN: 0-494-13973-0
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ISBN: 0-494-13973-0

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Abstract

This dissertation investigated the prevalence and select ecological impacts of root grafting in lodgepole pine. To document the frequency of root grafting, groups of trees were hydraulically excavated and the root systems were examined. Results indicate that root grafting frequency was controlled by tree density, tree age and tree diameter; as these variables increased, the rate of grafting also increased. The greatest distance recorded between grafted trees was 109 cm, which suggests that grafting would not be a common occurrence in stands with less than 8,500 stems per hectare (based on even tree distribution).

We also examined whether root grafts can transfer sufficient carbohydrate reserves among trees to affect the vigour of grafted trees growing in light-limited environments. At each plot, one tree in a grafted pair and one non-grafted tree were shaded for one growing season. Results indicate that shaded trees had significantly lower carbohydrate reserves and smaller crowns relative to non-shaded trees. However, root grafts appeared to partially offset the effects of shading as grafted shaded trees had significantly higher root carbohydrate reserves than non-grafted shaded trees. This implies that root grafts can transfer enough photosynthate to affect the carbon economy of subordinate trees, which may allow grafted trees to persist longer under shaded conditions than non-grafted trees.

The longevity of live roots on snags and stumps grafted to living trees and the impact of these roots on the growth of live trees were investigated. Grafted tree pairs

composed of one live tree and one snag and leave trees that had their grafted partners cut two growing seasons previously were assessed. Results demonstrate that the percentage of live roots on snags or stumps grafted to living trees gradually decreased until virtually no live roots remained 15 years after death of the crown. Also, diameter increment in the living trees significantly increased following manual thinning, but was unaffected when the grafted partner died naturally. From these results, it appears that the improved growing conditions following thinning were more important for tree growth than the inheritance of a large neighbouring root system connected by a graft.

Acknowledgements

I would first like to thank my supervisors, Vic Lieffers and Simon Landhäusser, for their time, patience and dedication to mentoring graduate students.

Thanks also to my silviculture lab mates Heather Fish, Tara Mulak and Lee Martens for helping maintain morale and for their helpful advice on thesis and non-thesis related topics.

I would also like to thank my parents Alan and Jean Fraser, my sister Leah Fraser, and my main squeeze James Reid for persevering with me and offering me invaluable encouragement along the way.

I would like to thank Ian Kwantes, Erin Smith, Anna Brown, James Reid, Mike Klaczek, Jennifer Hofman, Pak Chow and Monica Molina-Ayala for field and laboratory assistance. I would also like to thank Brian MacDonald from Blue Ridge Lumber (West Fraser Mills) for help in locating study sites.

Finally, I would like to thank West Fraser Mills, Weldwood of Canada, Weyerhaeuser Company, the Killam Trust, the University of Alberta, the Department of Renewable Resources and the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding.

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Chapter I: General introduction

It is generally assumed that coniferous trees exist as discrete individuals that compete with each other for critical resources such as light, water and nutrients. In fact, competition for these resources is generally considered to control the rate and pattern of mortality in forest stands (e.g.: Mohler et al. 1978, Knox et al. 1989, Nilsson et al. 2002). However, in many forests root grafts can form between different trees and it has been suggested that root grafts may alter the normal competitive forces in forest stands (Kuntz and Riker 1956, Bormann 1962, Eis 1972).

Root graft formation:

True root grafts involve the union of cambium, phloem and xylem tissues of two or more previously separate roots (Graham and Bormann 1966). In general, root grafts form when there is firm physical contact between adjacent tree roots (Bormann and Graham 1959, Eis 1972). Considering that roots are firmly anchored in the soil, any increases in root diameter will cause roots to become tightly pressed together and over time, a pressure point can develop on adjacent roots (Bormann and Graham 1959, Eis 1972). Based upon studies that have examined the process of graft formation at the cellular level, it appears that the cambium cells will eventually produce callus tissue at this pressure point (Bormann and Graham 1959, Eis 1972). Ultimately, the callus tissue breaks down the bark on the adjacent roots and the annual rings near this section become deformed (Eis 1972). The time required to break down the bark on adjacent roots varies, with younger roots typically requiring less time than older roots (Eis 1972). Following elimination of the intervening bark, the cambium cells from the two roots unite and

vascular continuity is established between the roots (Bormann and Graham 1959, Eis 1972).

Root graft formation and site characteristics:

The frequency of root graft formation does not appear to be affected by most site characteristics. Indeed, it has been shown that the occurrence of root grafting is independent of both soil texture (Bormann and Graham 1959, Eis 1972, Stone 1974) and topography (Eis 1972). Root grafts appear to be as common on sites with sandy soils as on sites with clay soils and similarly frequent on ridges, slopes and depressions (Bormann and Graham 1959, Eis 1972). However, graft formation has been linked to factors that increase the frequency of contact of large roots among trees, such as close inter-tree spacing (Schultz and Woods 1967, Eis 1972) and shallow rocky soils (Eis 1972).

Root grafts and transport of resources:

Previous research has indicated that root grafts are able to transfer water (Schultz and Woods 1967, Stone and Stone 1975), minerals (Kuntz and Riker 1956, Bormann 1966, Shultz and Woods 1967), carbohydrates (Bormann 1961, Bormann 1966), hormones (Bormann 1966), herbicides (Bormann and Graham 1959, Bormann and Graham 1960, Eis 1972), dyes (Bormann and Graham 1959, Stone 1974) and micro-organisms (Eis 1972, reviewed by Epstein 1978) among grafted trees. In general, resources move from healthy dominant trees to more suppressed trees or stumps (Kuntz and Riker 1956, Eis 1972), likely due to differing concentration gradients, although transport from stumps to healthy trees has also been observed (Bormann 1966, Schultz

and Woods 1967). It has been noted that dyes and radioactive tracers do not always move into all trees or stumps directly grafted to the injected trees (Bormann and Graham 1959, Graham 1960, Bormann 1966). Consequently, it has been suggested that certain factors may influence the amount and timing of solute movement through root grafts. Indeed, it has been proposed that greater movement across root grafts occurs when grafted trees or stumps are in close proximity (Schultz and Woods 1967), trees are connected by a large graft area (Graham 1960) and weather conditions are conducive to high transpiration rates (Bormann and Graham 1959, Schultz and Woods 1967).

Once critical resources like water and/or carbohydrates are transferred across root grafts, it appears that these solutes are readily transported vertically, but that there is only limited lateral movement (Bormann 1961, Bormann 1966). This lack of lateral transport is strongly evident in the xylem tissues as it has been reported that there was only 0.2 cm of lateral movement for every 1 m of upward tracer movement in suppressed Eastern white pine (*Pinus strobus* L.) trees grafted to more dominant trees (Bormann and Graham 1959). Transport in the phloem is also primarily vertically oriented; however, there appears to be a greater capacity for radial movement in these tissues (Bormann 1966). As a result, it has been suggested that the translocation of carbohydrates across root grafts is of greater importance than the movement of water as carbohydrates would likely be more accessible to the receptor tree (Bormann 1966).

Root grafts and forest stand dynamics:

Since root grafts can transfer critical resources like carbohydrate reserves among trees, this suggests that stand dynamics in heavily grafted forests may be altered relative

to the processes taking place in less heavily grafted forests. Considering that mortality in the juvenile stages is typically concentrated among the smallest trees (Mohler et al. 1978, Cannell et al. 1984, Kenkel et al. 1997), it is possible that the transfer of photosynthate from dominant trees to those in lower canopy positions could allow these weaker individuals to persist longer than if not grafted to a vigorous tree. Also, it is possible that the consistent transfer of resources from a dominant to subordinate tree could reduce the growth and vigour of the dominant tree, however this has not yet been tested.

Other pathways for belowground resource transfer:

While considerable research has demonstrated that critical resources can be transferred among trees via root grafts, it has also been shown that carbohydrates, water and nutrients can be transferred among trees that are not grafted together. Indeed, there is extensive evidence suggesting that mycorrhizal networks can transfer carbon and nutrients between plants (e.g.: Bjorkman 1960, Finlay and Read 1986, Simard et al. 1997). Mycorrhiza is a "symbiotic, non-pathogenic association between a plant root and a fungus" (Newman 1988) and it is possible for the fungal hyphae from one plant to colonize the roots of adjacent plants to form a common mycorrhizal network whereby many plants can be inter-connected (Simard and Durall 2004). As with root grafting, the magnitude of resource transfer between plants connected via a mycorrhizal network has been shown to be affected by sink strength (e.g.: Finlay and Read 1986, Ritz and Newman 1986, Simard et al. 1997). For instance, in a study of carbon transfer between Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and paper birch (*Betula papyrifera* Marsh.) seedlings, a net transfer of 9.5% of total carbon was recorded when the receptor Douglas fir seedlings were deeply shaded compared to a net transfer of only 2.7% when

both the donor and receptor trees remained under ambient light conditions (Simard et al. 1997). Consequently, mycorrhizal carbohydrate transfer could affect forest stand dynamics similarly to root graft transfer. It is possible that competition-related mortality could decline in areas with many mycorrhizal connections as carbohydrates transferred from more dominant to suppressed individuals could allow weaker plants to persist (Simard and Durall 2004).

It has also been suggested that inter-plant resource transfer can occur between individuals with no direct cellular contact (e.g.: Bormann 1957, Woods and Brock 1964). Several studies have suggested that water and nutrients can be exuded into the soil and absorbed by the roots of adjacent plants. Indeed, Bormann (1957) conducted a study whereby plants were grown with two intact root systems, with each root system growing in a separate pot. A second plant grew alongside one of the root systems and the single root system was watered while the root system growing with the second plant was not (Bormann 1957). The results of this study indicate that sufficient water was transferred from the well watered root system to the unwatered root system and eventually to the soil surrounding the unwatered root system to delay or prevent wilting in the second plant (Bormann 1957). Consequently, other pathways appear to operate in combination with root grafts to transfer critical resources among plants and trees.

Other mechanisms that may affect forest stand dynamics:

Substantial research has been carried out in an attempt to determine the mechanisms that influence forest stand dynamics and one factor that appears to play an

important role is hydraulic conductivity. It has been noted that sapwood permeability is substantially reduced in suppressed trees compared to those in more dominant canopy positions (e.g.: Keane and Weetman 1987, Shelburne and Hedden 1996, Reid et al. 2003). However, it has also been demonstrated that suppressed trees have greater stem hydraulic supply capacities per unit projected leaf area relative to more dominant trees (Reid et al. 2003). This, combined with the fact that suppressed trees typically grow in a shaded environment where water demands are low, has lead to speculation that suppressed trees may be better able to supply water to the crown relative to trees in more dominant canopy classes (Reid et al. 2003). Consequently, suppressed trees may be better able to survive short-term water shortages (Reid et al. 2003), which could slow the rate of self-thinning in some forest stands.

Interestingly, site quality has also been proposed as a factor that may affect forest stand dynamics for many of the same reasons that were suggested for hydraulic conductivity. For instance, it has been shown that trees growing on poor sites tend to have reduced sapwood permeability (Shelburne et al. 1993, Shelburne and Hedden 1996) as well as smaller crowns and less leaf area relative to trees growing on better quality sites (Keane and Weetman 1987). Consequently, trees growing on nutrient poor sites may also be able to withstand short-term water shortages as these trees have reduced water demand and increased water transport capacity.

Root grafts and manual thinning:

Logically, the transfer of resources among trees via root grafts could have an impact on tree growth responses following manual thinning treatments. Indeed, it has been demonstrated that both above and belowground portions of stumps created during manual thinning can be kept alive through photosynthate transfer from grafted neighbouring trees (e.g.: Bormann 1966, Schultz and Woods 1967). The length of time that a stump can persist following partial cutting varies among species (Newins 1916, Bormann 1961, Lanner 1961), but there are reports of stumps persisting for up to 200 years following removal of the crown (Newins 1916). Based on these previous observations, it appears that species that form callus tissue over the exposed stump or snag surface soon after the death or removal of the crown, such as Douglas fir and true firs (*Abies* spp.), seem to be able to survive longer than those species that form only a resin cap on the exposed surface, such as Eastern white pine and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Newins 1916, Bormann 1961, Lanner 1961). It has been suggested that this callus tissue is resistant to fungal and insect attack, whereas the resin exudation merely delays the time of decay entry (Lanner 1961, Miller and Woods 1965).

It is currently unclear whether the transfer of resources from living trees to support stumps with live tissue affects the growth rates in the live grafted partners; there have been reports of significantly increased growth (Bormann 1966), decreased growth (Eis 1972) and no change in growth (Walters 1963) relative to non-grafted control trees. As a result, it is currently not clear whether the grafted stump root system provides the living tree with an increased absorptive root surface area or whether the stump and its

roots are merely a drain on the resources of the living tree. Also, the rate and pattern of root mortality on stumps grafted to living trees is currently unknown. It is possible that as the stump breaks down and decays, roots that are more distal to the graft with the live tree may be cut off from the supply of carbohydrates and this could lead to increased root mortality on the far vs. near side of the stump.

Lodgepole pine ecology:

Forests dominated by lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex Loud.) cover approximately 20 million hectares in Canada, primarily in Alberta and British Columbia (Lotan and Critchfield 1990), which makes up approximately 22% of the forested land in these provinces (Koch 1995). Lodgepole pine is a pioneer species that is generally very intolerant of shading and intense competition (Lotan and Critchfield 1990). This fire-adapted species carries persistent and serotinous cones and as a result, millions of seeds per hectare can be held in reserve for release following a stand-replacing fire (Perry and Lotan 1979). Consequently, lodgepole pine has the tendency to regenerate in very high densities following disturbances like wildfire and it is not uncommon for over 100,000 seedlings per hectare to establish in the first growing season (Smithers 1957).

Root grafts and lodgepole pine:

Lodgepole pine was selected for this study of root grafting because root grafting should be prevalent in this species as the roots of many different trees should readily come into contact with each other. Root grafting has been documented in many other

North American coniferous species, including tamarack (*Larix laricina* (Du Roi) K. Koch), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), red pine (*Pinus resinosa* Ait.), Douglas fir and eastern hemlock (*Tsuga canadensis* (L.) Carr.) (list compiled by Graham and Bormann 1966), however to date there have been no investigations into the effects of root grafting in lodgepole pine stands.

Research objectives:

The objectives of this dissertation were to investigate the prevalence and some of the potential biological impacts of root grafting in lodgepole pine stands. Specifically, the objectives of Chapter II were to document the prevalence of root grafting in lodgepole pine forests and to determine whether stand characteristics such as tree height, tree diameter, tree age or stand density affect the rate of root graft formation. The objective of Chapter III was to determine whether root grafts can transfer sufficient carbohydrate reserves from a source tree to a grafted sink tree to affect the vigour of grafted trees relative to non-grafted trees growing in similar environments. Chapter IV was designed to investigate the longevity and location of live roots on lodgepole pine snags and stumps grafted to living trees and to determine whether these live roots affected the diameter growth of living trees.

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Chapter II: Age, stand density and tree size as factors in root and basal grafting of lodgepole pine[1]

Introduction

Root grafting between trees of the same species has the potential to be both physiologically and ecologically important in forest stands. Previous research has indicated that grafted trees are able to transfer water (Schultz and Woods 1967, Stone and Stone 1975), minerals (Kuntz and Riker 1956, Bormann 1966, Shultz and Woods 1967), carbohydrates (Bormann 1961, Bormann 1966), hormones (Bormann 1966), herbicides (Bormann and Graham 1959, Bormann and Graham 1960, Eis 1972), dyes (Bormann and Graham 1959, Graham 1960, Horton 1969, Stone 1974) and micro-organisms (Eis 1972, reviewed by Epstein 1978) across grafts. This transfer of resources has the potential to affect rates of growth and mortality of grafted trees. For instance, it has been demonstrated that stumps and girdled trees can be kept alive by the transfer of water and/or carbohydrates across root grafts (Bormann and Graham 1959, Bormann 1961, Horton 1969, Eis 1972, Stone 1974, Dosen and Iyer 1979). In fact, Stone (1974) reported that red pine (*Pinus resinosa* Ait.) trees continued to grow in height and diameter 18 years after girdling by using resources transferred across root grafts from non-girdled trees. Such results suggest that trees in heavily grafted stands may behave less like independent individuals and more like a community (Kuntz and Riker 1956, Bormann 1962, Eis 1972). Considering that inter-tree competition may not occur in the strictest sense among grafted trees, it has been suggested that it may be necessary to reassess the

[1] A version of this chapter has been published in the Canadian Journal of Botany 83(8): 983-988.

accepted concepts of plant competition to incorporate the non-competitive relationships occurring among grafted trees (Kuntz and Riker 1956, Bormann 1962, Eis 1972).

It is generally accepted that root graft formation occurs when there is firm physical contact between roots (Bormann 1966, Eis 1972). The process of root graft formation has been examined at the cellular level and it appears that as roots increase in size and become tightly pressed together, the cambium cells produce callus tissue at the pressure point (Eis 1972). This callus tissue breaks down the bark on the overlapping roots, causing deformation of the annual rings. Eventually, the cambiums of the two roots unite and vascular continuity is established between the two roots (Eis 1972). Several studies of coniferous species have indicated that root graft formation is independent of both soil texture (Bormann and Graham 1959, Bormann 1962, Eis 1972, Stone 1974) and topography (Eis 1972). However, graft formation has been linked to factors that increase root density and thus, the amount of contact between roots, including close inter-tree spacing (Schultz and Woods 1967, Eis 1972) and shallow rocky soils (Eis 1972). However, these previous root grafting studies have usually been carried out in isolated stands with relatively uniform tree ages, sizes and densities. Consequently, the current grafting data are generally confined to specific stands and more general relationships about the conditions necessary for grafting to occur cannot be reliably inferred. In order to predict which stands will be heavily grafted and to more fully assess the potential impacts of root grafting on forest stand dynamics, a more thorough understanding of the types of stand characteristics that lead to root graft formation is necessary.

To date, there have been no replicated studies that have directly investigated whether root grafts occur in lodgepole pine (*Pinus contorta* Dougl. Ex. Loud.) stands. Lodgepole pine is a pioneer species that can regenerate in very high densities following lethal disturbances like wildfire (Lotan and Critchfield 1990). As a result, roots would be in close contact in these dense forests and this may lead to root grafting. However, it is currently not known which stand characteristics may encourage the formation of root grafts in lodgepole pine forests.

The objectives of this study were to investigate the prevalence of root grafting in lodgepole pine forests and to determine whether stand characteristics affect the rate of root graft formation.

Materials and Methods

Site description

All sites were located in pure lodgepole pine stands in the Lower Foothills Natural Ecoregion near Swan Hills, Alberta (54° 45' 12N; 115° 42' 14W) or Hinton, Alberta (53° 23' 60N; 117° 34' 60W). Study sites were located in 20 dense fire-origin stands that were dispersed over four large areas. All sites had been undisturbed since initiation. Study trees ranged from 0.1-12.5 m in height, 0.17-13.3 cm in diameter and 2-46 years of age. Plot densities ranged from 8,118- 335,994 stems per hectare (sph) (Table 2-1). All sites were flat or slightly inclined with soils that ranged from sand to clay loam in texture.

Field assessments

Forty plots, each containing 10 contiguous trees, were randomly selected in the summers of 2003 and 2004. Plot diameters ranged from 0.6 to 3.9 m. All trees in each plot were numbered and the distance and bearing from a central tree to the nearest surrounding nine trees were recorded. Each tree was then cut down and measured for height and a stem section was removed (10 cm above ground level) for the determination of diameter and age. Each plot area was hydraulically excavated with a Wajax Mark III® fire pump (Tyco Suppression Systems, Pembroke, Bermuda) to a depth of ~ 30 cm so that all lateral roots were exposed. Following excavation, the plot area was carefully examined for root grafts. If root grafts were observed, the grafted unit (containing tree stumps, roots and grafts) was removed intact from the ground and transported back to the laboratory for analysis. If no root grafts were observed on a root system, no further assessments were made.

Laboratory assessments

Intact grafted units were assessed and the distance from each graft to the parent tree and the diameter of each grafted root was measured (average of two perpendicular measurements on the root immediately adjacent to the graft). All grafts were removed from the root system with a reciprocating saw and were serially sectioned with a band saw into 0.25-0.50 cm thick sections (Fig. 2-1). Graft sections were examined and total root age at the graft point, age of the graft, time for graft formation, root age at graft initiation and root diameter at graft initiation was recorded.

Age of the graft was measured by counting the number of continuous rings shared by the grafted roots at the centre of the graft. Time for graft formation was determined

by counting the number of rings growing into the callus tissue between the grafted roots. Once the rings were continuous between the two grafted roots, it was determined that the graft had formed.

Based on the spatial data collected at each site, stem maps were created for each plot with version 2000i of AutoCAD® (Autodesk Inc., San Rafael, CA). From these stem maps, distances between grafted trees within the plot were measured.

Statistical analysis

The relationships between the number of root grafts per m^2 in each plot and the average tree density, tree age, height and diameter in each plot were analyzed with multiple linear regression. Regression relationships were developed for root grafts (graft between lateral roots) and basal grafts (graft involving at least one stem/taproot) separately, however as these two categories of grafts appeared to have similar relationships for measured stand variables, the two types of grafts were combined into a single regression equation. Plot tree density did not conform to the assumptions of normality or equality of variance, so these values were *ln* transformed.

Logistic regression was used for the analysis of the relationships between the percentage of grafted trees and tree age, and secondly, between the percentage of grafted trees and distance between trees. As the root and basal grafts also demonstrated the same relationships in these analyses, the two types of grafts were combined for both relationships. For ease of presentation, these data were presented as bar graphs with age class and distance class categories.

The graft distance from the parent stem, root age at graft initiation and root diameter at graft initiation were tested using chi-square analysis. Release 8.1 of SAS® (SAS Institute Inc. Cary, NC) was used for all analyses and a significance level of α=0.05 was used for all response variables. The logistic regression analysis was done with PROC LOGISTIC in SAS®.

Results

The number of root grafts per m^2 was best predicted by tree density and stem diameter using multiple linear regression ($P<0.001$, $R^2=0.536$). The following equation was developed for the relationship:

[2-1] Grafts per m^2= -30.10+2.32 *ln*(tree density)+0.026 stem diameter (Table 2-2)

The proportion of grafted trees also increased with increasing tree age as indicated by logistic regression ($P<0.001$, $R^2=0.223$). Approximately 73% of trees in the 26-30 year age class supported at least one root or basal graft (Fig. 2-2). Also, the frequency of grafting decreased with increasing distance between trees ($P<0.001$, $R^2=0.194$); grafting occurred between approximately 20% of trees less than 10 cm apart and this rate decreased as distance between trees increased to less than 0.5% of trees grafted that were greater than 60 cm apart (Fig. 2-3). However, if trees younger than 15 years of age (these young trees were <25% grafted, Fig. 2-2) were removed from the analysis, grafting occurred between approximately 63% of trees less than 10 cm apart and 31% of trees less than 30 cm apart.

Both root and basal grafts appear more prevalent close to the stem of the parent tree (root and basal grafts $P<0.001$, chi-square analysis); 85% of root grafts and 97% of basal grafts occurred within 20 cm of the stem (Fig. 2-4). Further, graft initiation tended to occur early in stand development. Approximately 63% of root grafts and 46% of basal grafts had initiated by the time the roots were 10 years old and 96% of root grafts and 92% of basal grafts had established by age 20 (root and basal grafts $P<0.001$, chi-square analysis, Fig. 2-5). Also, grafting was typically initiated when coarse roots were relatively small; 81% of root grafts and 55% of basal grafts were initiated when roots were less than 30 mm in diameter and 95% of root grafts and 75% of basal grafts initiated when roots were less than 50 mm in diameter (root and basal grafts $P<0.001$, chi-square analysis, Fig. 2-6).

Overall, 32% of study trees supported at least one root or basal graft within the plot areas. However, if the trees younger than 15 years (these young trees were <25% grafted, Fig. 2-2) were removed from the analysis, the rate of grafting increases to 46% of study trees within the plot areas. Grafted trees supported 1-14 root and/or basal grafts, but averaged 2.6 grafts (±2.1 S.D.) per tree. The average time for graft formation was 2.3 years (±1.5 S.D.) for root grafts and 3.1 years (±1.7 S.D.) for basal grafts. Age of grafts ranged across the sites from 1-21 years, but averaged 4.6 years (±3.2 S.D.) for root grafts and 6.7 years (±4.4 S.D.) for basal grafts. If the ages of grafts are subtracted from tree ages in the current study, functional grafts (xylem and phloem contact, Fig. 2-1) appear to form at 4-45 years of age. However, on average functional grafts tended to establish when trees were 19.4 years old (±8.2 S.D.).

Discussion

The rate of root and basal graft formation in lodgepole pine stands appears to be primarily influenced by stand density, tree diameter and tree age (Equation 2-1, Fig. 2-2, Fig. 2-3). In the present study, areas with greater stand densities, greater average tree diameters and higher tree ages had a greater frequency of grafting. While grafting occurred under a range of stand conditions, it is logical to expect a greater rate of grafting in stands with higher tree densities and diameters as these conditions would increase the probability of roots coming into contact. Further, as trees increase in size and age, roots would become increasingly woody and more firmly embedded in the soil. Therefore, pressure may be more likely to build up between immobile roots and root grafting may occur with greater frequency. This would be especially true close to stems as roots would be very immobile, have large diameters and presumably, relatively large radial growth increments. In fact, the present study indicated that 86% of all root grafts and 98% of all basal grafts occurred between trees less than 50 cm apart (Fig. 2-3) and that the majority of grafts formed within 20-30 cm of the stem (Fig. 2-4). The maximum distance between grafted trees measured in this study was 109 cm; therefore, we suggest that significant amounts of root grafting would not occur in stands with densities less than 8,500 sph (assuming even tree spacing). Previous studies of root grafting have generally examined stands with relatively uniform densities and ages so direct comparison between previous work and the present study is difficult. Nevertheless, root grafts have been recorded in Eastern white pine (*Pinus strobus* L.) stands ranging from 1,600 sph to 11,700 sph with stem diameters ranging from 6.6-19.8 cm (Bormann and Graham 1959). Grafts have also been observed at a variety of tree ages, ranging from one-year old loblolly pine (*Pinus*

taeda L.) nursery stock (Shultz and Woods 1967) to 71-year old Eastern white pine (Bormann and Graham 1959).

Root and basal grafts appear to initiate early in stand development. Both root and basal grafts are present in lodgepole pine stands by age 6-10, but they do not appear to affect more than 30% of trees until after age 20 (Fig. 2-2). On average, it appears that functional (full xylem and phloem contact) grafts establish when trees are 19-20 years old. Also, most grafts (95% of root grafts, 75% of basal grafts) in the present study began to form when roots were less than 50 mm in diameter (Fig. 2-6). The trends observed in this study may have been influenced by the relatively young stands (2-46 years old) we investigated. We cannot determine from our results whether grafts continue to form as stands age. It is possible that trees a metre or more apart may become joined by grafts as trees continue to increase in age and size. This may continue throughout the life of a stand because as trees mature, large roots capable of exerting significant pressure against other roots will form at distances greater than 10 cm from the base of the stem (Fig. 2-4). Thus, grafting may readily occur in stands with densities less than 8,500 sph if trees are mature and have large root systems. Further, it is possible that as stands age, the root systems of trees lost to above-ground competition-related mortality could be maintained through grafts, thus further increasing the size of an individual root system and increasing the probability of grafting between trees greater than 109 cm apart. However, as roots grow farther away from the tree base, they likely become less firmly embedded in the soil and the pressure required for root graft formation may not readily build up between roots. Nevertheless, the relationships in the present study are similar to

those observed in red pine where roots were 6-19 years of age and 0.7-3.4 cm in diameter at graft initiation (Armson and van den Driessche 1959).

The techniques used in this study may have underestimated the number of grafts present in the study areas. As we only measured grafts within specific plot areas, we did not account for grafts that involved plot trees but were located outside the plot boundaries. As a result, the number of grafts per tree and the total number of grafted trees may be underestimated. Also, the prevalence of grafting may have been further underestimated due to the very young trees measured in several plots. Trees less than 15 years of age have a very low probability of grafting (Fig. 2-2) and since 16 plots had an average tree age of less than 15 years, this may have further reduced estimates of grafts per tree and total grafts. However, we believe the relationships that incorporated grafts/m^2 (linear regression) to be accurate as this measurement is expressed on an area basis, as opposed to at an individual tree level.

Recognizing the stand characteristics that lead to root graft formation is important for a more complete understanding of the role of grafting in forest stands. It has been suggested that the concept of inter-tree competition as a central component of stand dynamics may need to be reassessed because trees in heavily grafted stands may behave more like a community than independent competitive individuals (Kuntz and Riker 1956, Bormann 1962, Eis 1972). It is possible that the transfer of water, minerals and carbohydrates among trees (see Introduction) could aid the growth and survival of small

weak individuals at the expense of the larger donor trees. However, more work is needed to clarify the possible impacts of root grafting on tree growth and mortality rates.

Table 2-1: Mean characteristics (averaged on a plot basis) of the trees in the 40 excavated plots.

Stand variable	Maximum	Minimum	Mean	Standard Deviation
Tree height (m)	12.5	0.1	4.6	2.6
Tree diameter (cm)	13.3	0.2	4.1	1.9
Tree age (years)	46	2	17.3	9.3
Tree density (sph)	335,994	8118	53,682	62,428

Table 2-2: Significance values for multiple linear regression analysis.

Variable	Parameter estimate	F-value	P-value
Intercept	-30.10	6.33	0.016
ln (Density)	2.31	5.12	0.030
Stem diameter	0.026	37.74	<0.001

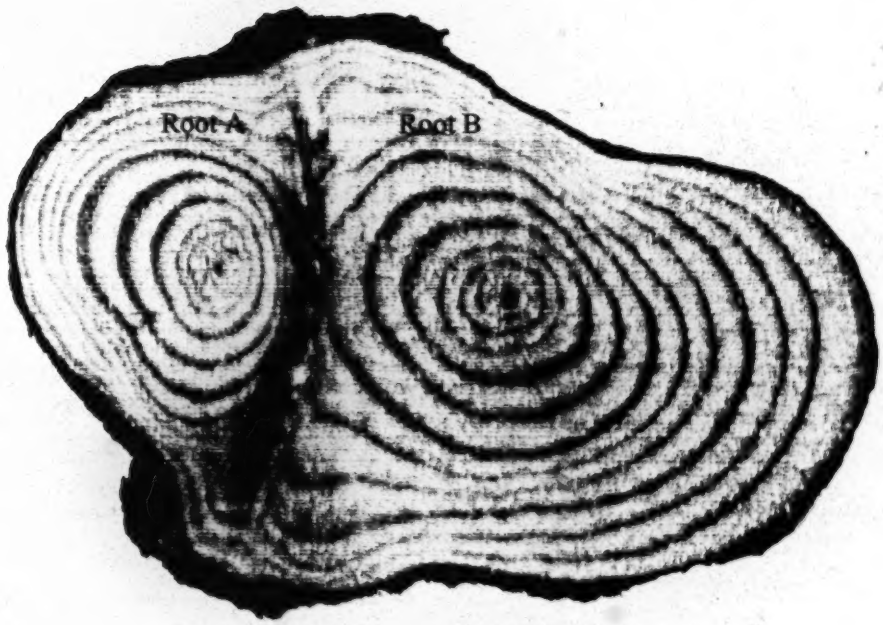

Figure 2-1: Cross-section of a five year old root graft. Note the joining of xylem and phloem layers and the central area with callus formation. In this photo, root A was 7 years old and root B was 8 years old at graft initiation. This graft took 3 years to form and has been functional (shared xylem and phloem) for 3 years.

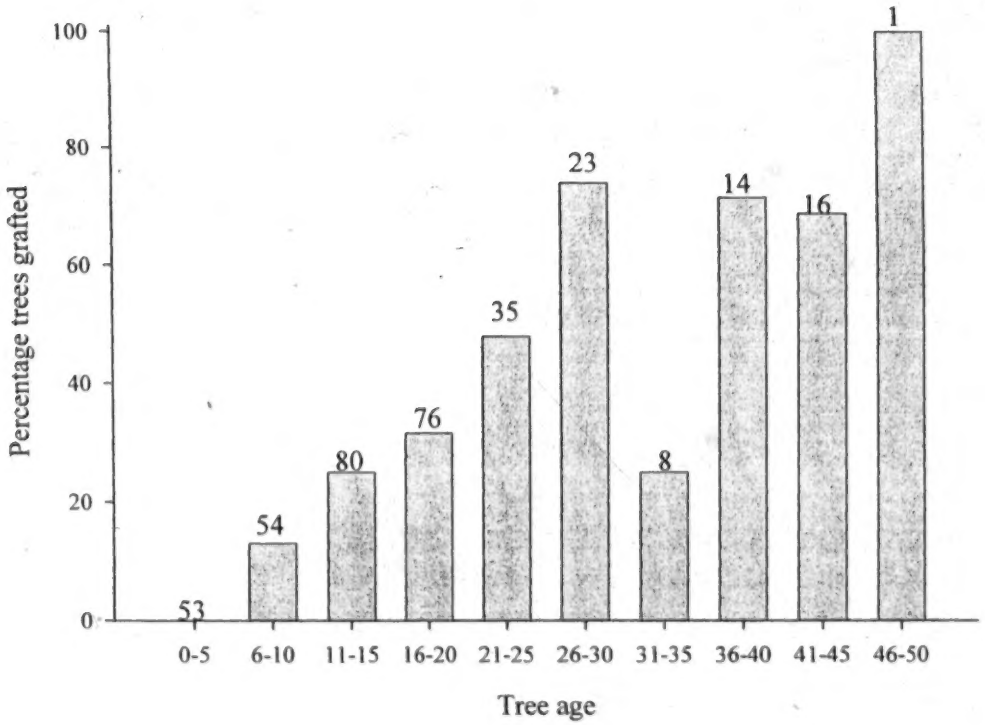

Figure 2-2: Percentage of trees with root/basal grafts in each age category. Percentages were calculated separately based upon the number of trees within each age class. Numbers above bars indicate total number of trees measured in each category.

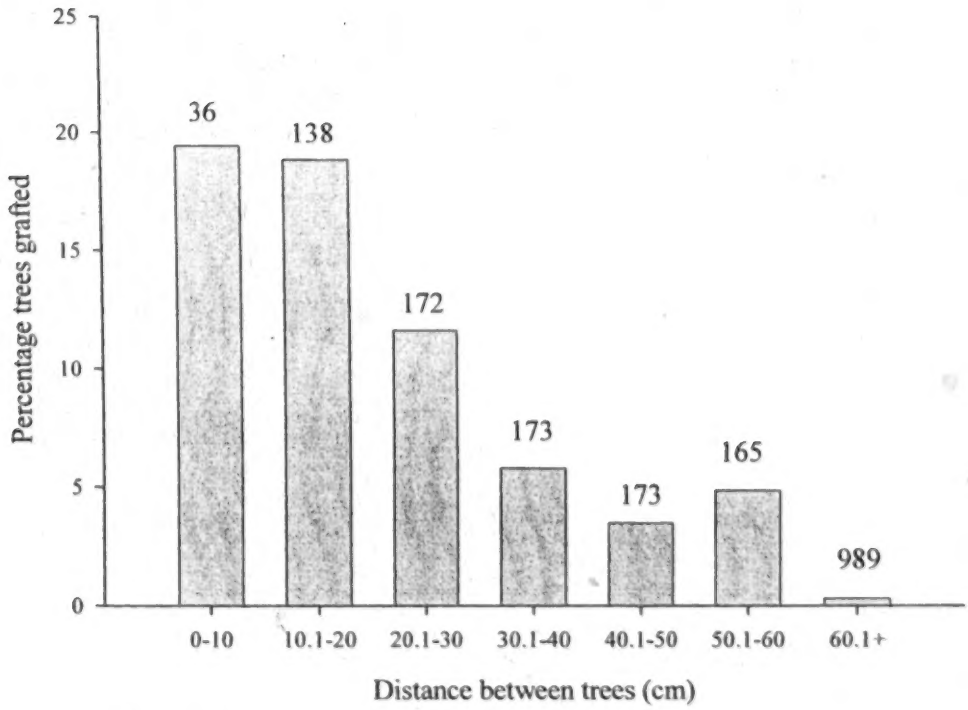

Figure 2-3: Percentage of trees with root/basal grafts in each distance category. Percentages were calculated separately based upon the number of trees within each distance category. Numbers above bars indicate total number of trees measured in each category.

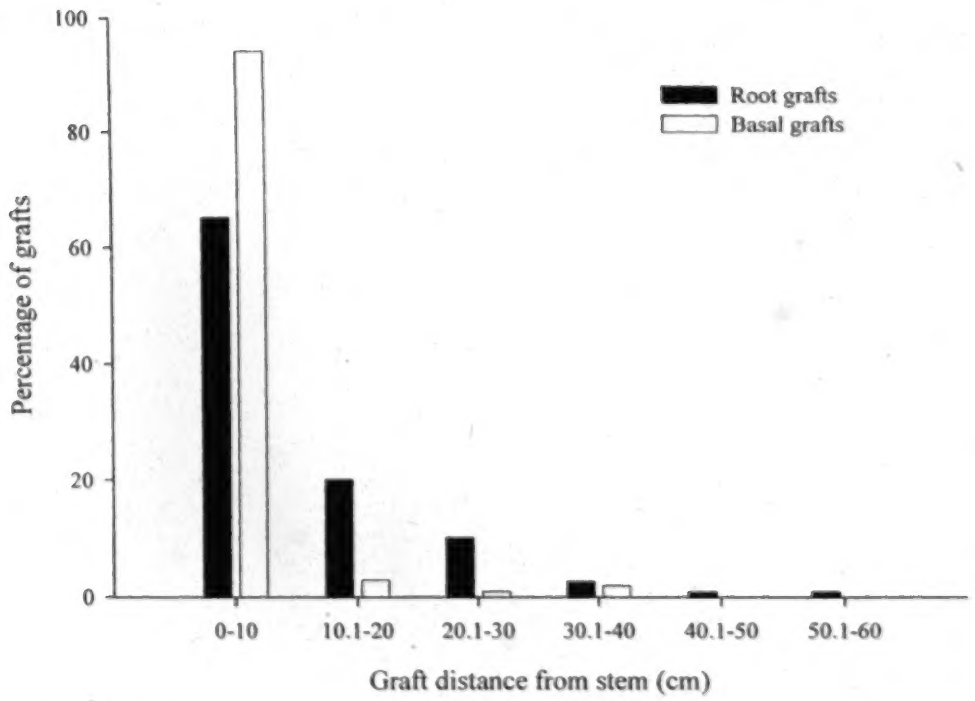

Figure 2-4: Percentage of all grafts found in the 40 plots, in relation to distance between graft and stem.

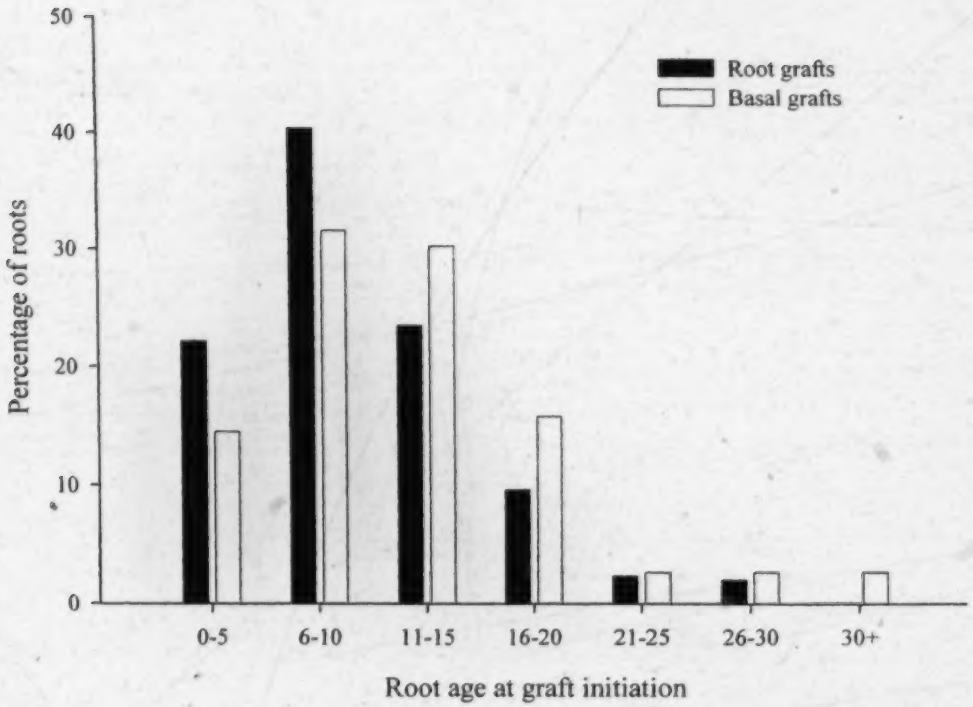

Figure 2-5: Percentage of all grafted roots found in the 40 plots, in relation to age at graft initiation.

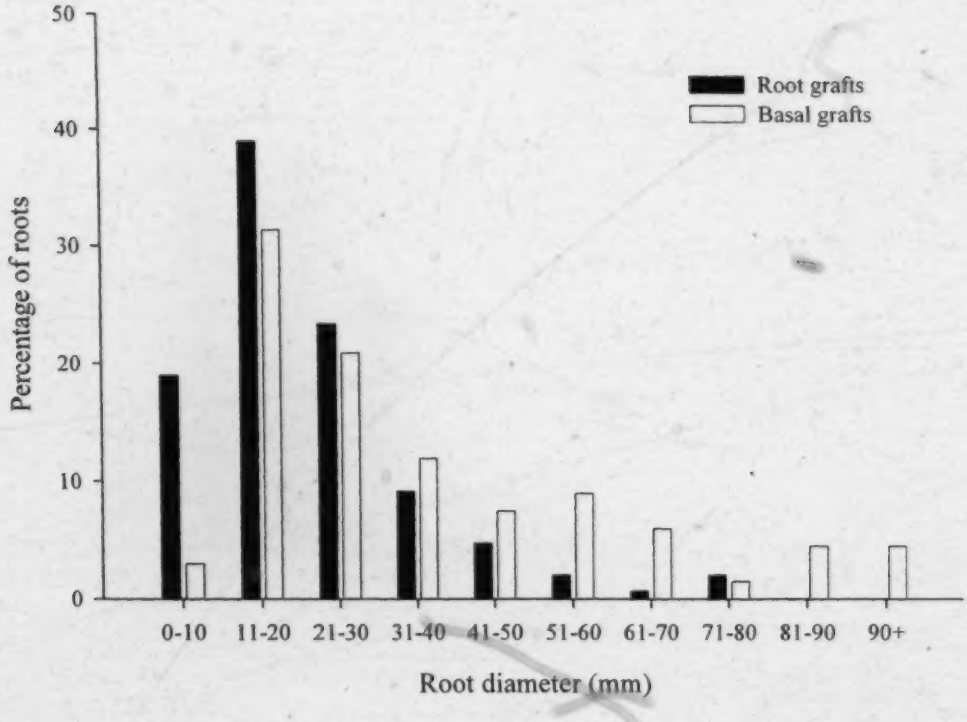

Figure 2-6: Percentage of all grafted roots found in the 40 plots, in relation to root diameter at graft initiation.

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Chapter III: Carbohydrate transfer through root grafts to support shaded trees[2]

Introduction

Following stand-replacing disturbances, some tree species establish at high densities and there is initially little competition among seedlings (Cannell et al. 1984, Kenkel et al. 1997). After establishment, individual growth rates diverge and trees begin to differentiate into crown classes (Knox et al. 1989, Nilsson et al. 2002). It is generally assumed that competition for critical resources (i.e.: light, water, nutrients) controls the rate and pattern of tree mortality in forest stands (e.g.: Mohler et al. 1978, Knox et al. 1989, Nilsson et al. 2002) and that mortality will be concentrated among the smallest individuals (Mohler et al. 1978, Cannell et al. 1984, Kenkel et al. 1997). Competition for light is usually considered to be asymmetric because taller trees are able to capture a disproportionate amount of the resource (e.g.: Knox et al. 1989, Berntson and Wayne 2000). As a result, smaller trees in dense stands generally perish due to lack of light.

It has been suggested that the presence of root grafts may alter normal competitive relationships among trees and influence forest stand dynamics (Kuntz and Riker 1956, Bormann 1962, Eis 1972). Previous research has indicated that water (Schultz and Woods 1967, Stone and Stone 1975) and carbohydrates (Bormann 1961, Bormann 1966) can be transferred across grafts. The quantity of water and/or carbohydrates that can be moved through root grafts is not known, but it has been demonstrated that intact trees can transport enough photosynthate to keep the root systems of girdled trees (Bormann 1966, Stone 1974) and stumps alive for years

[2] A version of this chapter has been submitted for publication in Tree Physiology

following exclusion of the photosynthesizing tops (e.g.: Bormann 1961, Schultz and Woods 1967, Eis 1972). Nevertheless, it is not clear whether these resources may be transferred to trees in inferior light environments such as might occur under asymmetric competition for light (Knox et al. 1989, Bernston and Wayne 2000).

We chose lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex. Loud.) for the study of root grafting because it commonly regenerates in extremely high densities following wildfire (Lotan and Critchfield 1990, Blackwell et al. 1992) and it can form extensive root grafts in dense stands greater than 15 years of age (Fraser et al. 2005). In juvenile stages these dense stands often have very poor crown differentiation and as a result, suppressed trees that normally should be removed via competition often persist and there may be little change in density over time (Blackwell et al. 1992, Reid et al. 2003). It is our research hypothesis that the transfer of resources through root grafts in lodgepole pine will help to support the continued existence of trees in inferior positions in dense stands.

The objective of this study was to determine whether root grafts can transfer carbohydrate reserves from an intact source tree to a grafted sink tree that grows in a light-limited environment, and to test whether this relationship affects the vigour of grafted trees relative to non-grafted trees growing in similar environments.

Materials and Methods

Site description

All study sites were located in three pure fire-origin lodgepole pine stands near Swan Hills, Alberta, Canada (54° 45' 12N; 115° 42' 14W). Trees in the study areas ranged from 3.0-6.1 m tall and 2.2-9.6 cm in stem diameter at 10 cm height and were 14-19 years old. All sites were flat or slightly inclined and soils were Grey Luvisols.

Field assessments

Eleven plots, each containing six trees were established May 4-6, 2004. Each plot area contained two grafted pairs of trees and two independent non-grafted trees. Based on earlier results (Fraser et al. 2005), two trees growing within 20 cm of each other had a high probability of being grafted while trees at least 80 cm apart were likely not grafted. The grafting status was verified at the end of the experiment (see below). The overall tree density of the plots was high (>8,000 trees per hectare), but tree pairs or single trees were at least 80 cm from their neighbours. All trees used in this study were in a dominant or co-dominant canopy position and both trees in a given grafted pair were of approximately equal height and stem diameter. Plot areas varied in size, but trees within the same plot were generally within 10-15 m of each other (depending on the distribution of grafted tree pairs). Plots were located at least 100 m apart and there were 3-4 plots in each of the three forest stands.

At each plot, the lowest intact live whorl of branches (determined by having green needles on all branches of the whorl) was identified and marked on each tree. Also, a branch (with needles) was removed from the mid-crown of each tree for carbohydrate analysis. One non-grafted tree and one "assumed grafted" pair of trees were randomly

selected in each plot and left undisturbed for the experimental period. The remaining trees were assigned to a shading treatment. However, only one tree in the grafted pair was shaded and the other half of the pair remained non-shaded throughout the experiment. Therefore, at each plot there was one non-grafted non-shaded tree (NN), one non-grafted shaded tree (NS), one grafted pair of trees where both trees were non-shaded (GNP) and one grafted pair of trees where there was one shaded (GS) and one non-shaded tree (GN) (Fig. 3-1).

For the shading treatment, black fibreglass insect screen (7 strands/cm) that blocked approximately 75% of the photosynthetically active radiation (measured with a Sunfleck® ceptometer, ITC International, Armidale Australia) was used. In order to evenly shade the study trees, the screen was sewn into two different sized cylinders: one approximately 4 m long and 1.5-2 m in diameter and the other approximately 1 m long and 30 cm in diameter. The larger cylinder covered the entire tree crown up to the base of the leader. The smaller cylinder loosely covered the leader, leaving approximately 30-40 cm of extra screen on top of the leader to allow for growth during the experiment. The insect screen was securely wrapped around each shaded tree with twine and the top of the small cylinder covering the leader was closed to ensure each tree would remain fully covered during the experimental period. Also, two Hobo H8® (Onset Computer Corporation, Bourne, Massachusetts, USA) temperature data loggers were located at each of three randomly selected plots (one data logger at mid-crown under the shading screen and one at mid-crown of a non-shaded tree) to record crown temperatures during the experimental period.

On August 30-31, 2004, following one growing season, all six trees in each plot were cut and the grafted/non-grafted status of all study trees was determined via root excavation. Also, at each plot the following measurements were taken: current leader increment and crown recession distance (distance between locations of lowest whorl of live branches in May vs. August). Further, a branch (with needles) at mid-crown, a non-grafted coarse root at least 3 cm long and a stem section (10 cm above ground level) were collected from each tree for carbohydrate, nutrient and stem analysis. The grafted stumps and large roots of each grafted tree pair was removed intact from the ground and transported to the laboratory for analysis.

Laboratory assessments

Stem diameter, tree age, the rate of stem diameter growth during the experiment (2004 *growth*) and the total stem diameter growth during the previous four years (2000-2003 *growth*) were determined for each tree from the stem sections that were collected. Relative stem growth rates (RSG) of all trees was calculated with:

$$[3\text{-}1] \quad RSG = \left(\frac{2004\ growth}{(2000\text{-}2003\ growth)/4} \right) \times 100\%$$

Further, all grafts were removed from the grafted root system with a reciprocating saw and were serial sectioned with a band saw into 0.25-0.50 cm thick sections. Graft sections were then examined and the age of the graft, the xylem area of the graft and the phloem circumference of the graft were measured and recorded.

Samples of branches collected from mid-crown (spring and fall collections), needles on branches collected at mid-crown (spring and fall collections) and coarse roots (fall collection only) were ground and total non-structural carbohydrate (TNC) concentrations were determined (Chow and Landhäusser 2004). Total nitrogen (N) and phosphorus (P) contents were also determined for the new needles that grew at mid-crown during the experimental period using Kjeldahl digestion (Bremner and Mulvaney 1979).

Statistical analysis

The relationship between shading and crown air temperature was tested as a completely randomized one-way ANOVA. The relationships between treatment type (GS, GN, GNP, NS, NN) and leader increment, crown recession distance, relative stem growth, branch, needle and root carbohydrate concentrations and new needle total N and P levels were analyzed as a randomized block 2x2 factorial design with 2 levels of grafting (grafted, non-grafted) and two levels of shading (shaded, non-shaded). As there were two trees in the GNP treatment at each plot and both trees received the same treatment, the data for these trees were averaged at the plot level. The GNP trees were included at each plot to determine whether there were any differences between non-grafted non-shaded trees and grafted non-shaded trees. As there were no statistical differences detected between GNP and NN trees for any response variables ($P>0.100$), the GNP trees were removed from the final analysis of variance. All data in this study conformed to the assumptions of normality and equality of variance. The root excavations following the end of the experimental period showed that three "grafted" pairs of trees (two GS-GN pairs and one GNP-GNP pair) were not actually grafted

together, so the number of replicates within each treatment was: 9 GS, 9 GN, 13 NS and 15 NN. In the randomized block factorial analysis, each plot was designated as a "block", so there were 11 blocks in this study.

The relationships between root, branch and needle carbohydrate concentrations and graft xylem area, graft phloem circumference, age of graft or the number of grafts connecting the two trees were analyzed with simple linear regression. If a tree pair was connected by more than one graft, the phloem circumferences/xylem areas of all grafts were summed so that the total phloem circumference/xylem area connecting two trees was analyzed. The linear regression analysis incorporated all grafted trees (GS, GN and GNP). Release 8.1 of SAS® (SAS Institute Inc., Cary, North Carolina, USA) was used for all analyses, multiple comparisons were done with linear contrasts and a significance level of α=0.05 was used for all response variables.

Results

The shading treatments did not have a significant effect on either daily maximum (P=0.133) or minimum (P=0.783) temperatures within the crown. On average, crown temperatures in shaded and non-shaded trees were within 1.1°C during the day and within 0.1°C at night from May-August.

There were no differences in total non-structural carbohydrate (TNC) concentrations in the needles or branches of the study trees at the beginning of the experimental period (P>0.351). Also, there were no significant interactions between

shading and grafting for any response variables tested in this study ($P>0.122$). Following the experiment, root TNC concentrations were significantly different among the four treatments ($P<0.001$, Fig. 3-2). The differences in root TNC concentrations appeared to be due to both shade ($P<0.001$) and the presence of root grafts ($P=0.032$); the non-grafted shaded (NS) trees had significantly lower root TNC concentrations than the grafted shaded (GS), grafted non-shaded (GN) and non-grafted non-shaded (NN) trees. On average, NS trees had root TNC concentrations of 3.5% compared to a mean of 5.1% in GS trees, 7.1% in GN trees and 6.9% in NN trees (Fig. 3-2).

Both branch and needle TNC concentrations were not different among the four treatments (branch $P=0.085$, needle $P=0.119$). Following the experimental period, branch TNC concentrations were 7.2% and needle TNC concentrations were 10.1%, regardless of shading or the presence of root grafts.

The amount of crown recession, the leader increments and relative stem growth (RSG) rates were all different among the four treatments (all $P<0.001$). For these three variables, the shading treatment significantly reduced crown and stem growth (crown recession $P<0.001$, leader increment $P<0.001$, RSG $P=0.007$, Table 3-1), but the presence of root grafts had no effect (crown recession $P=0.298$, leader increment $P= 0.703$, RSG $P=0.936$, Table 3-1).

Total nitrogen and phosphorus contents in the new needles were not significantly different among the five treatments (nitrogen P=0.060, phosphorus P=0.723). Total N averaged 1.35% and total P averaged 0.17% across all five treatments.

Both graft xylem area ($P<0.001$, $R^2=0.850$) and graft phloem circumference ($P=0.005$, $R^2= 0.291$) increased with increasing graft age (Fig. 3-3A and 3-3B). Neither xylem area nor phloem circumference appeared to affect the differences in sugar or starch concentrations between grafted pairs of trees ($P>0.135$). However, when corrected for the average tree volume of the grafted pair, the differences in root starch concentrations between GS and GN trees decreased with increasing ratios of graft phloem circumference: average tree volume ($R^2=0.584$, $P=0.017$, Fig. 3-4). This relationship was only observed when GS and GN trees were analyzed; in GNP trees, root starch concentrations were not affected by the ratio of graft phloem circumference to average tree volume of the grafted pair ($P=0.974$). Also, no relationships were observed between the number of root grafts connecting two trees, graft xylem area and/or graft phloem circumference and branch sugar or starch, needle sugar or starch or root sugar concentrations.

Discussion

Our results indicate that trees in inferior positions can gain resources from adjacent trees through root grafts. Indeed, total non-structural carbohydrate (TNC) concentrations in coarse roots were approximately 30% greater in grafted shaded (GS) trees relative to non-grafted shaded (NS) trees (Fig. 3-2). The largest impact of the grafts

was observed in the maintenance of greater TNC levels in the roots of shaded trees with grafts compared to shaded trees without root grafts (Fig. 3-2). The effects of root grafts on crown growth and foliar carbohydrate concentrations were less clear; there was a marginally non-significant trend for less crown recession (P=0.076) and non-significant trends for greater annual ring growth and greater leader increments in grafted shaded trees compared to non-grafted shaded trees (Table 3-1). However, the overall impacts of grafts were generally less evident on above-ground parts than at the root level. Given that the root system was the first resource sink to be in contact with the carbohydrates obtained from grafted neighbours, it is likely that few carbohydrates were passed on to the more distal organs above ground. A similar relationship has been observed in herbaceous plants grown in nutrient poor soils; the root system sequestered the majority of available nutrients and passed few resources on to the rest of the plant (reviewed by Clarkson 1985).

Since there were declines in foliar and root carbohydrate levels in shaded trees, especially in the non-grafted shaded trees, it is clear that the deep shading treatment (see photosynthetic light response curves by Landhäusser and Lieffers 2001) allowed relatively little photosynthesis to occur. It appears that the size of the phloem connection joining many of the trees was not sufficient to conduct enough carbohydrate reserves to satisfy the demands of the root system as well as the more distal organs at the top of shaded trees. Indeed, it appears that the size of graft was important in determining how much photosynthate was passed between trees (Fig. 3-4) and in most cases, it is likely that grafts were too small to deliver the carbohydrates needed to completely maintain

these large and heavily shaded neighbours. If the shaded neighbour was proportionately smaller than the non-shaded tree, as would be the case in asymmetric competition, the grafts may have been better able to support the shaded tree.

By maintaining the roots of shaded trees, it appears that root grafts had a major impact on the carbohydrate economy of shaded neighbours. To put this in perspective, lodgepole pine growing in light-limited conditions have root/shoot ratios that are approximately double that of most coniferous trees growing in the boreal forest (Pearson et al. 1984, Comeau and Kimmins 1989, Cairns et al. 1997) and this indicates that a disproportionate amount of resources are allocated to the root system at the expense of shoot growth when light levels are low (Pearson et al. 1984, Comeau and Kimmins 1989, Vanninen and Mäkelä 2005). Because the root TNC concentrations were greater in grafted shaded (GS) trees, the carbohydrates produced by these shaded trees would be more likely to be allocated to crown growth, which may allow grafted trees to persist longer in shaded conditions.

We expected to observe a parasitic relationship between the grafted shaded and grafted non-shaded tree pairs where the shaded neighbour would have a negative effect on the growth and root carbohydrate supply of the GN trees. However, we could not detect any significant growth reductions in the GN trees over one growing season. Instead, it appears that there was a relationship between grafted shaded and grafted non-shaded trees where the GS trees benefited from the relationship and the GN trees were largely unaffected. This result is surprising given the apparent carbohydrate transfer to

the root system of the shaded trees (Fig. 3-2). Nevertheless, it is possible that the increased absorptive surface area of the grafted root system may have improved the water supply to the foliage of non-shaded trees, as the shaded trees likely had low stomatal conductance due to the artificial boundary layer and low light levels. Also, the photosynthetic efficiency of GN trees may have been increased due to the high sink strength of the grafted shaded neighbour and grafted root system (e.g.: Neales and Incoll 1968, Herold 1980, Myers et al. 1999, Pieters et al. 2001).

From this study, it appears that root grafts may affect stand dynamics in forest stands. Our results indicate that grafted trees growing in light-limited environments were partially supported by carbohydrates transferred across root grafts from their more vigorous partners. Also, our results suggest that there were no significant growth reductions in GN trees following the transfer of resources to their shaded partner. Consequently, normal asymmetric competitive forces may be less important in stand dynamics in heavily grafted forests. Instead, we suggest that the size hierarchies that normally develop when trees grow in close proximity to one another and compete for light (e.g.: Knox et al. 1989, Kenkel et al. 1997, Nilsson et al. 2002) may develop at a much slower rate than is typically observed for independent trees. Even a moderate transfer of resources to a subordinate tree could reduce competitive asymmetry, especially when the transfer occurs year after year. Generally, mortality is greatest among the most suppressed individuals (see Introduction); however, suppressed trees that are grafted to a more vigorous partner may persist longer, especially if connected with a

large graft. Thus, root grafting could partially explain the slow self-thinning of high density lodgepole pine stands.

Table 3-1: Leader increment, crown recession distance and relative stem growth rates for each treatment type following four months. The treatments were: grafted shaded (GS), grafted non-shaded (GN), non-grafted shaded (NS) and non-grafted non-shaded (NN) trees. Numbers in parentheses represent the standard error of the mean. Numbers with different letters are significantly different at the 95% confidence level.

Treatment	Leader increment (cm)	Crown recession distance (cm)	Relative stem growth rate (%)
GS	14.0 (±1.5)^{A}	15.7 (±7.9)^{C}	76.6 (±11.2)^{F}
GN	36.8 (±2.2)^{B}	0.0 (±0.00)^{D}	98.6 (±13.1)^{EF}
NS	12.3 (±2.3)^{A}	36.4 (±11.1)^{C}	70.4 (±10.52)^{F}
NN	33.2 (±2.5)^{B}	0.0 (±0.00)^{D}	112.6 (±11.3)^{E}

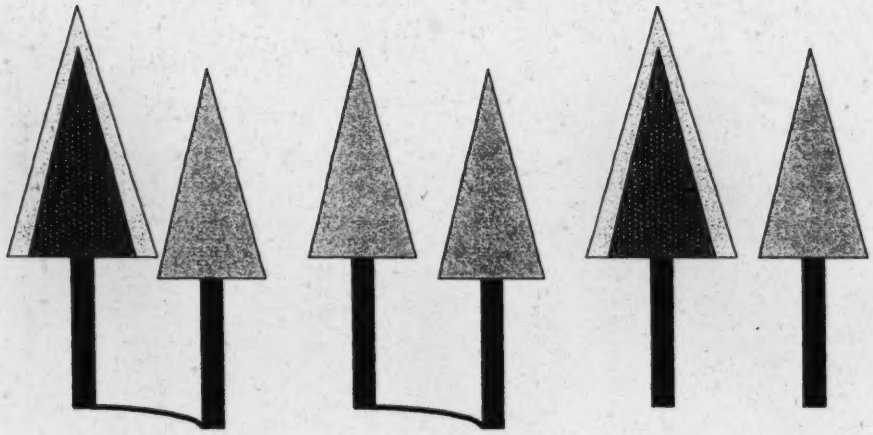

Figure 3-1: Diagram depicting different treatments present at each plot. Lines connecting two trees indicate root grafts. Covers over trees indicate shading treatment. Treatment abbreviations are: GS- grafted shaded, GN- grafted non-shaded, GNP- grafted non-shaded pair, NS- non-grafted shaded, NN- non-grafted non-shaded.

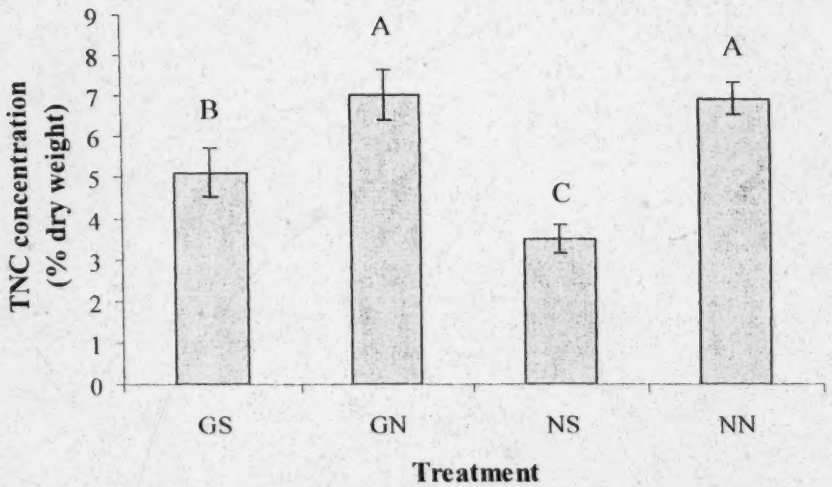

Figure 3-2: Total non-structural carbohydrate (TNC) concentrations (sugar + starch) in August 2004 for trees in each type of treatment. The treatments were: grafted shaded (GS), grafted non-shaded (GN), non-grafted shaded (NS) and non-grafted non-shaded (NN) trees. Sugar and starch values were analyzed separately. Bars represent the standard error of the mean and bars with different letters are significantly different at the 95% confidence level.

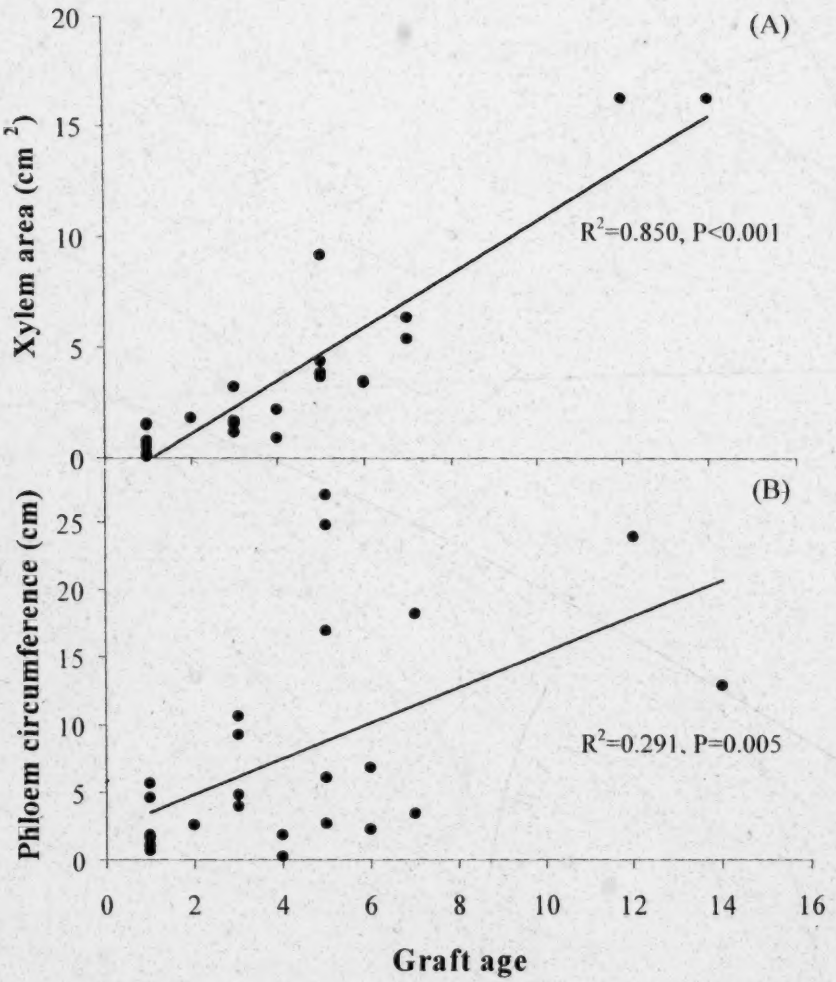

Figure 3-3: Relationship between graft age and common xylem area (A) and graft age and common phloem circumference (B).

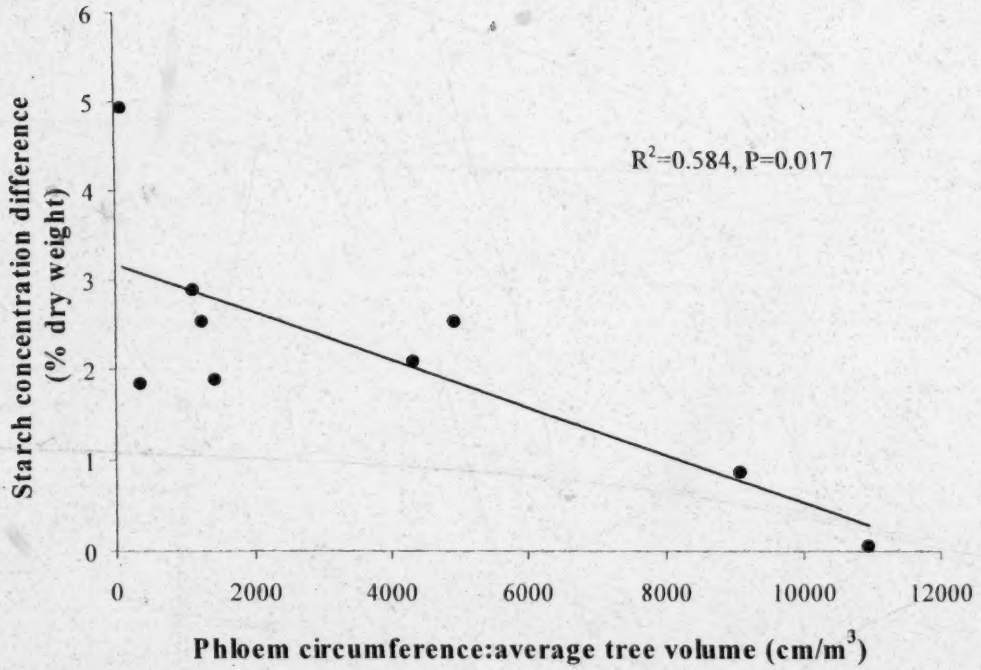

Figure 3-4: Relationship between the ratio of phloem circumference to average tree volume of the grafted pair (cm/m^3) and the difference in starch concentrations between grafted pairs of trees where one tree was shaded (GS trees) and one was non-shaded (GN trees).

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Chapter IV: The persistence of living roots on lodgepole pine snags and stumps grafted to living trees[3]

Introduction

Living roots on snags and stumps are a common occurrence in many coniferous forest stands (e.g.: Bormann 1961, Lanner 1961, Eis 1972, Stone 1974). Following the death of individual trees (either from natural causes or manual thinning), the base of the stem and root systems of snags and stumps can be kept alive through the transfer of carbohydrates via root grafts from living neighbouring trees (e.g.: Bormann 1966, Schultz and Woods 1967). Root reserves from non-grafted roots appear to play only a limited role in the maintenance of living stumps and snags; stumps not grafted to living trees can persist for up to one growing season after removal of the photosynthesizing tops (Bormann 1961), but survival beyond one year has always been attributed to photosynthate transferred across root grafts from living trees (e.g.: Bormann 1961, Lanner 1961, Schultz and Woods 1967, Eis 1972, Stone 1974).

There have been several studies that have assessed the survival rates of above-ground portions of snags and stumps following removal of the crown (e.g.: Newins 1916, Bormann 1961, Lanner 1961, Schultz and Woods 1967, Eis 1972). However, there have been no replicated studies that have investigated the longevity or mortality patterns of the root systems of stumps or snags grafted to living trees. It is also currently unclear whether the transfer of resources to living stumps or snags affects the diameter growth of intact living grafted partners. There have been reports of significantly increased growth (Bormann 1966), decreased growth (Eis 1972) and no change in growth (Walters 1963)

[3] A version of this chapter has been submitted for publication in the Annals of Forest Science

in living trees grafted to living stumps relative to non-grafted control trees. Also, previous studies have not differentiated between the effects of snags and stumps on living trees. Considering that snags usually form slowly over time, they may drain resources from their living grafted partner for several years as the crowns and stems slowly die. Conversely, stumps are formed suddenly (via manual thinning) and are symptomatic of more destructive disturbance thus, the above-ground parts would not be a potential sink for resources.

Lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex Loud.) was selected for this study because it has been previously shown to readily form root grafts, especially after 15 years of age (Fraser et al. 2005). Also, lodgepole pine is a shade intolerant species that has a tendency to form extremely dense single-species stands following natural disturbances (Lotan and Critchfield 1990). Therefore, many lodgepole pine stands are manually thinned during the juvenile stages (Lotan and Critchfield 1990). As a result, it is likely that numerous lodgepole pine stands are composed of living trees grafted to at least one snag or stump with live roots.

The objectives of this study were to determine the longevity and location of live roots on lodgepole pine snags and stumps grafted to living trees and to determine whether these live roots affect the diameter growth of adjacent living trees.

Materials and methods

Natural mortality study

This study used plots established within a large, pure fire-origin lodgepole pine forest near Hinton, Alberta (53° 23' 60N; 117° 34' 60W). Live trees in the study area averaged 8.1 cm in stem diameter (range: 5.7-11.8 cm, measured at 10 cm height) and 45.1 years of age (range: 39-54 years, measured at 10 cm height). Dead standing trees (snags) in the study area averaged 4.9 cm in stem diameter (range: 2.8-7.0 cm, measured at 10 cm height), 31.9 years of age (range: 22-39 years, measured at 10 cm height) and had been dead for an average of 14.7 years (range: 8-23 years, measured at 10 cm height) (see below). Plots were located on flat or slightly inclined terrain and soils were Brunisolic Grey Luvisols.

Twenty plots were established during August 2004. Each plot contained one living tree and one snag connected with a root graft. Trees were within 30 cm of each other and were presumed to be grafted together prior to the death of the snag. All plots were located at least 20 m apart. The root system of each tree pair was manually excavated and the grafting status was verified. The grafted snags and large roots were removed intact from the ground and transported to the laboratory for analysis.

Stem diameter and tree age were measured on all trees on a stem section taken at 10 cm height. Tree ring widths were measured on the stem sections with a Parker Instruments® stage micrometer (Vickers Instruments York, England) and dissecting microscope. Starting with the outermost ring, each ring was counted and rings that were narrower than average were noted. Calendar years were then assigned to rings in the

snags by comparing the ring patterns of living trees and their dead grafted partners at each plot (Stokes and Smiley 1968, Yamaguchi 1991). From this analysis, the year of death was established for all snags (Fig. 4-1). Also, all grafted roots were removed from the grafted root system, glued to a board and then serial sectioned with a band saw into 0.25-0.50 cm thick sections. The sections were either sanded or carefully shaved with a razor blade prior to examination of the annual rings. Graft sections were examined and the age of the graft, the area of the xylem across the graft and the circumference of the phloem across the graft were measured and recorded.

Tree ring increment (TRI) of the stem was also determined for each live tree. To calculate TRI, annual ring widths were plotted for each calendar year (Fig. 4-2). The greatest growth peak in the living tree that occurred at least ten years prior to the death of the snag was identified. An exponential function was then fitted to each data set starting at this growth peak and ending the year that the snag died. This exponential function was used to extrapolate the expected ring widths after the death of the snag (Fig. 4-2). Tree ring increment was calculated by dividing the observed ring widths by the expected ring widths for the two years immediately before and after the death of the snag (Dang and Lieffers 1989).

Also, the positions of all live roots relative to the graft were recorded on each snag. Live roots had moist xylem, phloem and cambial tissues and were marked immediately following excavation.

Manual thinning study

All study plots were established within three pure fire-origin lodgepole pine stands near Swan Hills, Alberta (54° 45' 12N; 115° 42' 14W). Trees in the study area averaged 5.7 cm in stem diameter (range: 2.2-12.5 cm, measured at 10 cm height) and 19.9 years of age (range: 16-30 years, measured at 10 cm height). Sites had less than 10% slope and the soils were Grey Luvisols.

Eight plots, each containing 3-6 trees, were established in May 2003. Plots were located in areas with clumps of trees with high stem densities (>25,000 sph) because these areas have been shown to have a high probability of root grafting (Fraser et al. 2005). Clumps were separated from adjacent trees by at least 1 m. At each plot, a dominant or co-dominant leave tree was selected and the surrounding trees were cut at 10-20 cm height with a brush saw. Trees selected as leave trees were healthy and had good form. Plot sizes varied, but were generally <2 m^2 and all plots were located at least 20 m apart.

In August 2004, two growing seasons after the thinning treatment, all plot areas were hydraulically excavated with a Wajax Mark III® fire pump (Tyco Suppression Systems, Pembroke, Bermuda) to a depth of approximately 30 cm so that all lateral roots were exposed. Following excavation, the grafted/non-grafted status of all plot trees was determined. The stumps and large roots of all trees grafted to the leave tree were removed intact from the ground and transported to the laboratory for analysis. Any stumps not grafted to the leave tree were eliminated from the study. Also, eleven dominant/co-dominant non-grafted control trees were selected from the area surrounding

the study plots; these trees were cut and the stem sections were transported to the laboratory.

Stem diameter, tree age and tree ring widths were measured and recorded on the leave trees, control trees and the stumps of trees removed during the manual thinning treatment. The position of all living roots on each stump was also recorded. Further, TRI was calculated for the leave trees and the non-grafted control trees in the same manner as described above. All grafts were removed from the grafted system and were serial sectioned into 0.25-0.50 cm thick sections so that graft age, graft xylem area and graft phloem circumference could be determined.

Immediately following excavation, samples of living roots from leave trees, control trees and stumps were collected. All root samples were dried, ground with a Wiley mill and total sugar and starch concentrations were determined (Chow and Landhäusser 2004).

Statistical analysis

The relationship between the percentage of live roots on the snag or stump and time since the death of the snag or stump and the circumference of the phloem across the graft was analyzed with both simple linear and multiple regressions. Also, the relationships between TRI and stem diameter of the snag or stump, age of the snag or stump, area of the xylem across the graft, circumference of the phloem across the graft, age of the graft and the percentage of live roots on the snag or stump were analyzed with multiple linear regression. The relationships between the age of the graft and graft xylem

area and graft phloem circumference were analyzed with simple linear regression. If a tree pair was connected by more than one graft, the xylem area and phloem circumference of all grafts were summed so that the total xylem area or phloem circumference connecting two trees was analyzed.

The data describing the positions of the living roots on the snags or stumps were analyzed with chi-square analysis. The root sugar and starch concentrations in leave trees, stumps and control trees were analyzed as a completely randomized one-way ANOVA and the changes in TRI before and after the death of a grafted partner were analyzed with paired t-tests.

Data from both the natural mortality and manual thinning studies were combined for all regression analyses. However, the two studies were analyzed separately in the chi-square analysis, the one-way ANOVA and the t-tests. All data in both the natural mortality and manual thinning studies conformed to the assumptions of normality and equality of variance. Release 8.1 of SAS® (SAS Institute Inc. Cary, NC) was used for all analyses, multiple comparisons were done with lsd tests and a significance level of α=0.05 was used for all response variables.

Results

The percentage of live roots on snags or stumps grafted to a living tree was correlated with the time that the snag or stump had been dead (Fig. 4-3) and the circumference of the phloem across the graft (Fig. 4-4). The percentage of live roots on

snags or stumps grafted to intact living trees was predicted well by a combination of these two variables in multiple regression ($P=0.035$, $R^2=0.273$).

[4-1] % live roots=17.80 -0.72 time since death +0.52 graft phloem circumference

In the natural mortality study, 83% of live roots on the snag were located within 90° of the root graft with the living tree compared to 61% in the manual thinning study (Fig. 4-5). Consequently, significantly more live roots were maintained close to the graft in the natural mortality study ($P=0.005$), while two years after manual thinning there were no significant differences in number of living roots on the near vs. far side of the stump ($P=0.140$, Fig. 4-5).

Following the death of roots directly grafted to the roots of living trees, the living root appeared to distinctly wall-off the xylem connecting it to the dead root (Fig. 4-6). In some circumstances, there was some staining of living roots through the grafted area, but there was no evidence of decay.

Tree ring increment (TRI) of the leave trees in the manual thinning study significantly increased following removal of the surrounding grafted partners ($P=0.002$, Fig. 4-7). Prior to thinning, leave tree TRI averaged 1.00 and this value increased to 1.35 in the two years following thinning. Over the same two time periods, the TRI of control trees was not significantly different from the TRI of leave trees in the two years prior to thinning ($P=0.212$, Fig. 4-7), but was significantly lower than the thinned plots in the two

years immediately following thinning (P=0.005, Fig. 4-7). In the natural mortality study, TRI in the living trees was not significantly affected by the death of their grafted partner (P=0.205), increasing from 1.00 to 1.04 after the death of the partner.

Both graft xylem area (P<0.001, R^2=0.189) and graft phloem circumference (P<0.001, R^2=0.210) increased with graft age (Fig. 4-8A and 4-8B). However, no significant relationships were detected between TRI and diameter of the snag or stump, age of the snag or stump, graft xylem area, graft phloem circumference, age of the graft or the percentage of live roots on the snag or stump (P>0.100).

In the manual thinning study, starch concentrations in the roots of leave trees and control trees were significantly greater than in living roots on stumps (P=0.016). On average, starch concentrations were 2.7 times greater in roots from leave trees and control trees relative to those from stumps (2.7% vs. 1.0%, Fig. 4-9). However, sugar concentrations in the live roots of leave trees and control trees were not significantly different from the sugar concentrations in stump roots (P=0.286); two years following thinning, sugar concentrations averaged 3.7% (Fig. 4-9).

Discussion

Our results indicate that the proportion of live roots on snags or stumps gradually declined over time and that virtually no live roots remained approximately 15 years after death of the crown (Fig. 4-3). Previous studies have assessed the longevity of above-ground portions of stumps and snags following partial cutting or other disturbances and

have reported variable results for different species. In general, it appears that species that form callus tissue over the exposed stump or snag surface soon after death, such as Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and true firs (*Abies* spp.), seem to be able to survive longer than those species that form only a resin cap on the exposed surface, such as Eastern white pine (*Pinus strobus* L.), ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Newins 1916, Bormann 1961, Lanner 1961) and lodgepole pine (this study). It has been suggested that this callus tissue is resistant to fungal and insect attack and eventually forms a bark layer, whereas the resin exudation merely delays the time of decay entry (Lanner 1961, Miller and Woods 1965). We did not observe any callus tissue formation in either our natural mortality or manual thinning studies, and based on previous work, callus tissue formation appears to be rare in most pine species (Newins 1916, Bormann 1961, Lanner 1961, Schultz and Woods 1967). Considering that the stump is the critical junction point for the graft to access more distal roots, a rotten stump would break the transport of carbohydrates and/or water through the stump and the growing tips of more distal roots would be cut off from the living tree. This theory of how grafted roots die is further strengthened by the fact that there was little evidence of pathogens invading the graft itself prior to the final death of the snag or stump root, suggesting that the final cause of death was more distal than the graft itself (Fig. 4-5). The partial discolouration of the xylem in some grafts is likely not pathogenic as the discoloured tissue in the live roots was always uniform. Interestingly, even after several years of graft connection, the boundary between live and dead tissue appeared to be defined by the lines of the original roots (Fig. 4-6).

In the present study we observed more live roots on snags or stumps that were connected to living trees with a large graft (large phloem circumference across the graft). In fact, virtually all snags or stumps with live roots were grafted to a living tree with a graft phloem circumference of at least 20 cm (Fig. 4-4). Logically, grafts with larger phloem pathways should be able to transport greater quantities of carbohydrates to the snag or stump root system. Indeed, previous research has indicated that greater quantities of starch were transferred from non-shaded trees to their deeply shaded partners via root grafts when the phloem circumference of the graft was large (Chapter III).

In our manual thinning study, live roots were relatively evenly distributed around the stumps two years after thinning (Fig. 4-5). However, in our study of natural mortality 83% of live roots were located within 90° of the junction of the grafted root to the tree base and only 17% were on the far side of the tree base (Fig. 4-5). These results complement those obtained by Bormann (1961) who previously reported that the majority of living tissue visible on Eastern white pine stumps was located on the side of the stump closest to the living grafted partner. It has been shown that there is very little lateral movement of solutes in xylem tissues of stumps grafted to living trees (Bormann 1961, Bormann 1966). Further, it has been suggested that in many cases, root grafts between lodgepole pine trees have too little contact to transfer significant amounts of carbohydrates between trees (Chapter III). Therefore, it is unlikely that the root or stump tissue distal to the graft would receive adequate carbohydrates from the living tree for long-term survival. . .

It is still not clear whether the inheritance of a large grafted root system had an impact on the growth of the leave trees in the manual thinning study. The fact that tree ring increments of leave trees grafted to cut trees significantly increased following manual thinning (Fig. 4-7) could be related to either the benefits of capturing more roots, or to the increased light and soil resource availability following thinning (e.g.: Alexander 1960, Bella and De Franceschi 1982, Yang 1998) or a combination of both. Conversely, when mortality occurred naturally and the dead trees remained as snags, there was little apparent benefit for the residual tree. In this case, the death of a single intermediate tree via natural causes would only provide a small increase in the availability of resources to the remaining trees. Nevertheless, if the grafted roots of the dying tree were beneficial to the residual tree, we would expect to see a positive growth response in the residual tree as water and nutrient availability should increase. However, tracer movement in the xylem tissues of stumps grafted to living trees has been considered slow and inefficient (Bormann 1966), which suggests that water uptake by these roots may be minimal. Conversely, movement of carbohydrates from the living tree to support a relatively large root system on the dead tree could easily overwhelm any positive effects of increased water uptake associated with these roots.

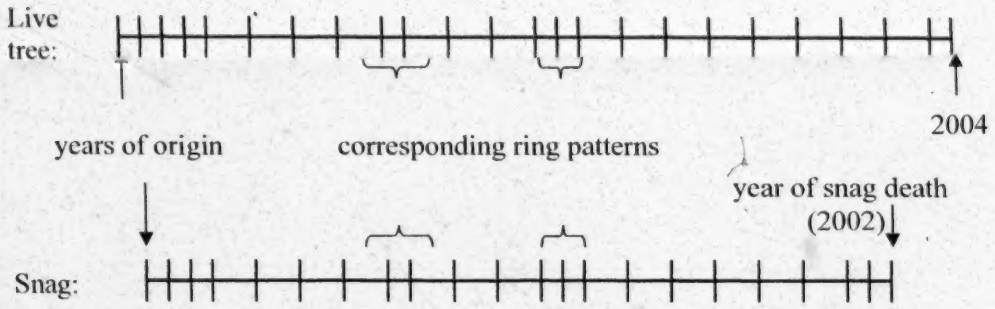

Figure 4-1: Diagram of cross-dating procedure. Note how corresponding ring patterns are matched up between the live tree and the snag so that the years of snag origin and death can be determined based upon the known year of live tree death (2004).

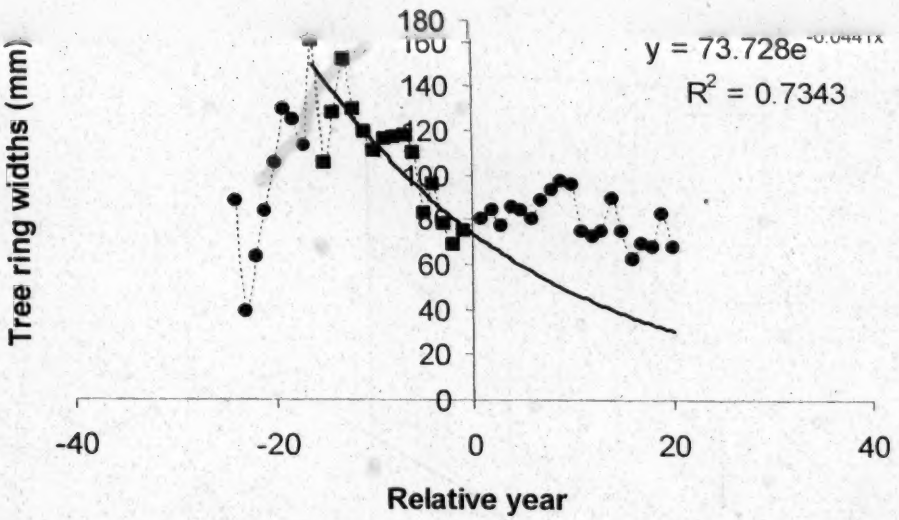

Figure 4-2: Relationship between tree ring widths in a live tree grafted to a snag or stump and the relative year of snag or stump death for one sample site. Relative year 0 denotes the year of snag or stump death. The circles represent actual ring growth throughout the life of the live tree. The squares represent the actual ring growth from the growth peak that occurred at least ten years prior to the death of the snag or stump and ending the year of snag or stump death. The solid line is an exponential function that was fitted to the data from the growth peak to the year of snag or stump death and this line has been extrapolated beyond the year of snag or stump death to calculate tree ring increment.

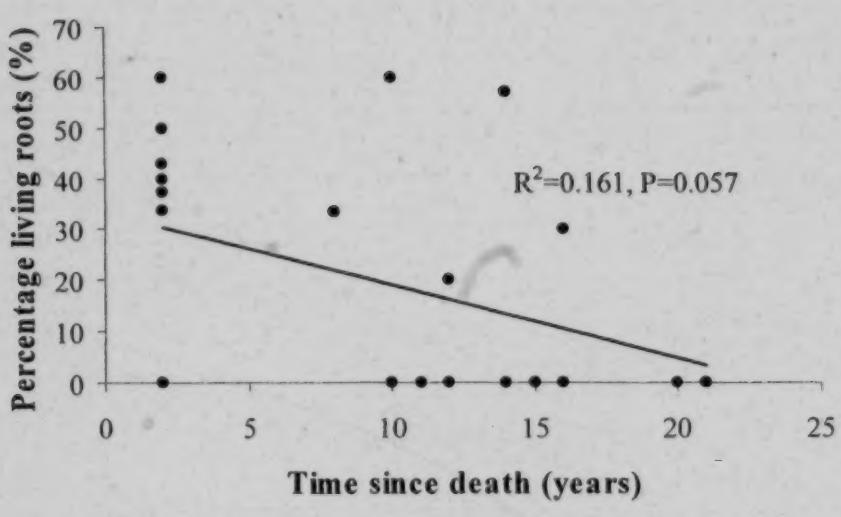

Figure 4-3: Relationship between the percentage of living roots on snags or stumps that were grafted to a living tree and the time since the death of the snags or stumps.

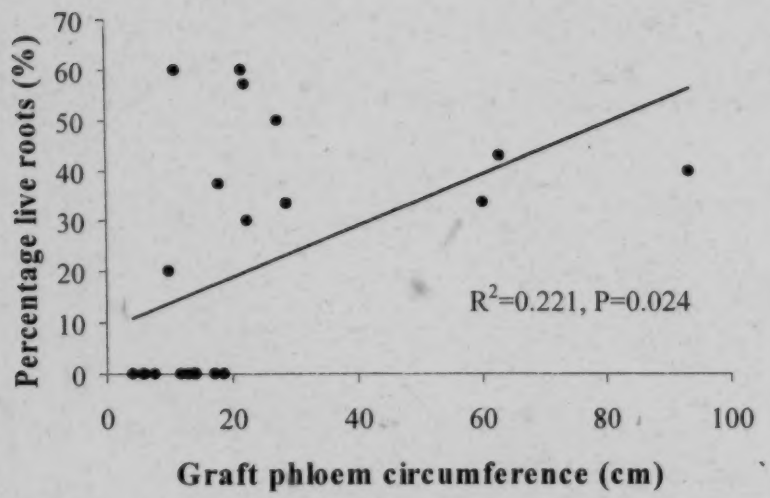

Figure 4-4: Relationship between the percentage of living roots on snags or stumps and the circumference of the phloem connection of the graft connecting the snag or stump to a living tree.

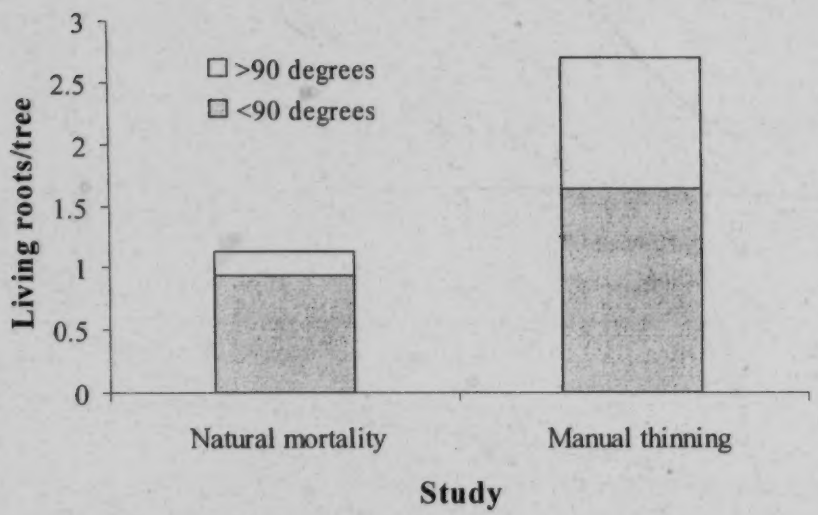

Figure 4-5: Number of living roots per tree on snags and stumps in the natural mortality and manual thinning studies. Living roots were located either >90° or <90° from the root graft connecting the snag or stump to a living tree.

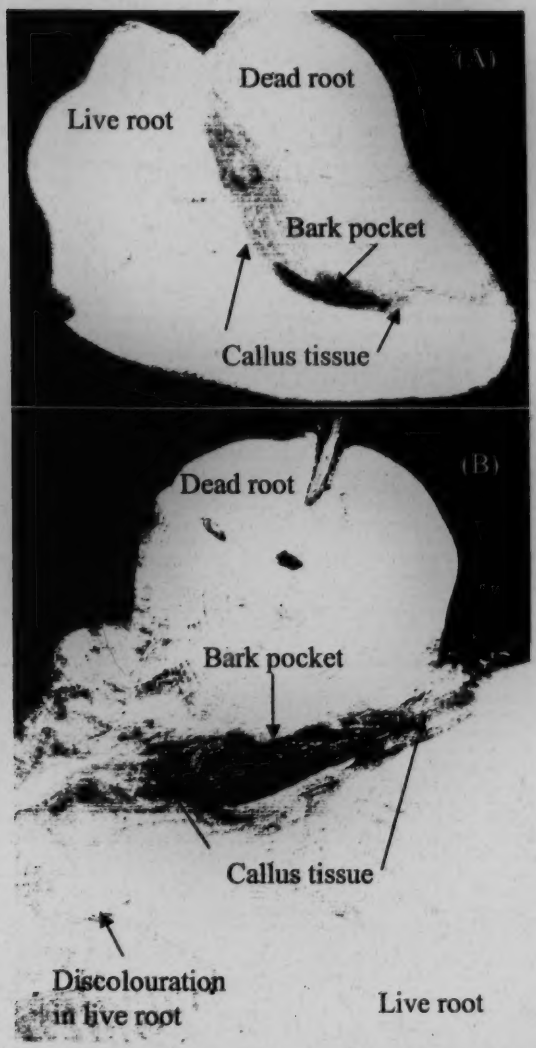

Figure 4-6: Photographs of root grafts between live and dead roots where there was no discolouration in the live root following the death of the dead root (A) and where there was some discolouration in the live root following the death of the dead root (B). The bark pocket and callus tissue denote the point of graft formation.

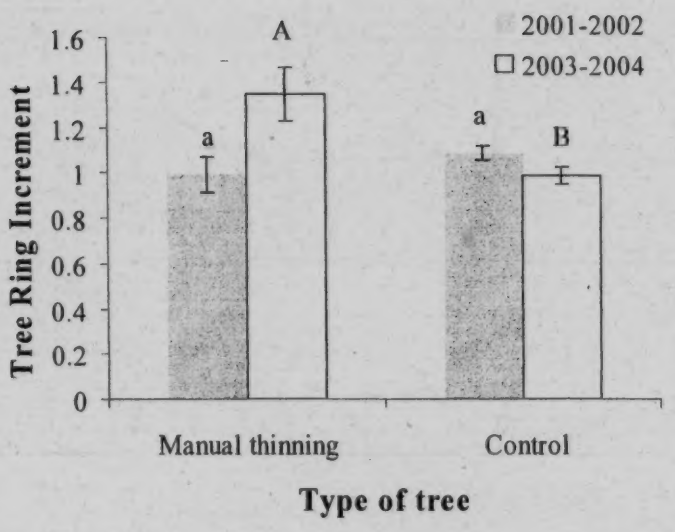

Figure 4-7: Tree ring increment during 2001-2002 (before thinning) and 2003-2004 (after thinning) in live trees that had their grafted partners removed (manual thinning) and in trees that received no treatment (control). Bars represent the standard error of the mean. Bars with different letters are significantly different at the 95% confidence level. Data for 2001-2002 and 2003-2004 were analyzed separately.

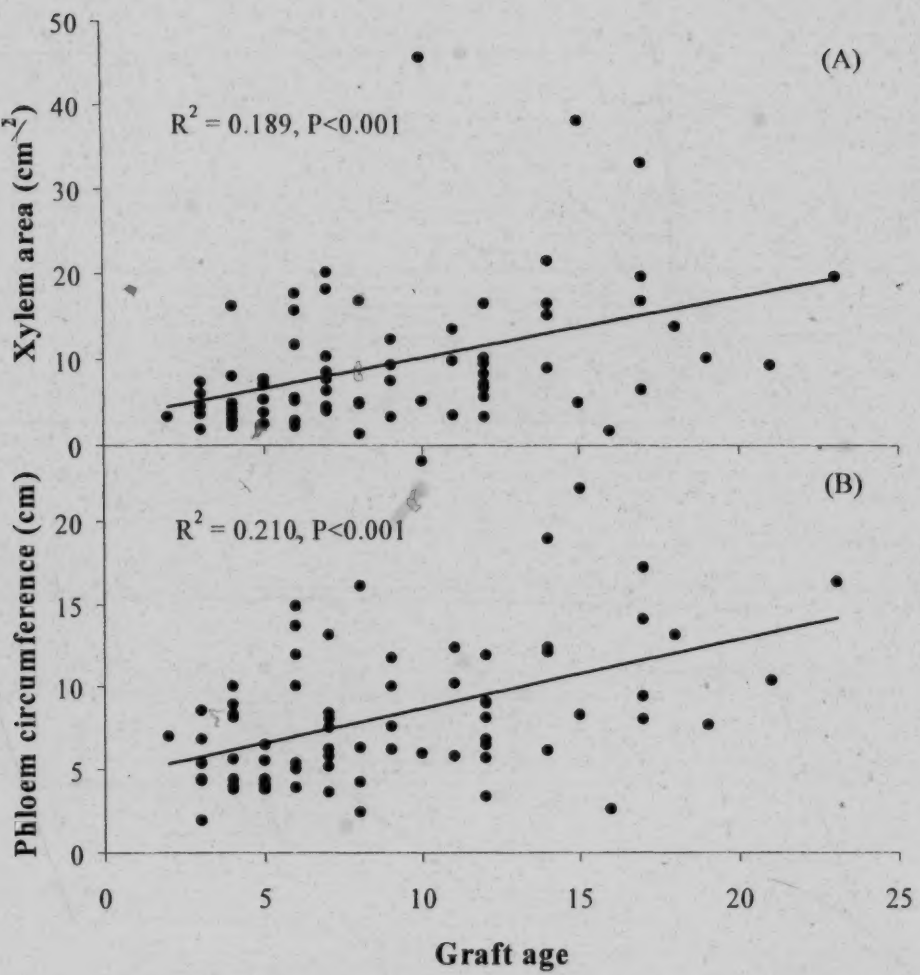

Figure 4-8: Relationship between graft age (years since grafting) and graft xylem area (A) and phloem circumference across the graft (B).

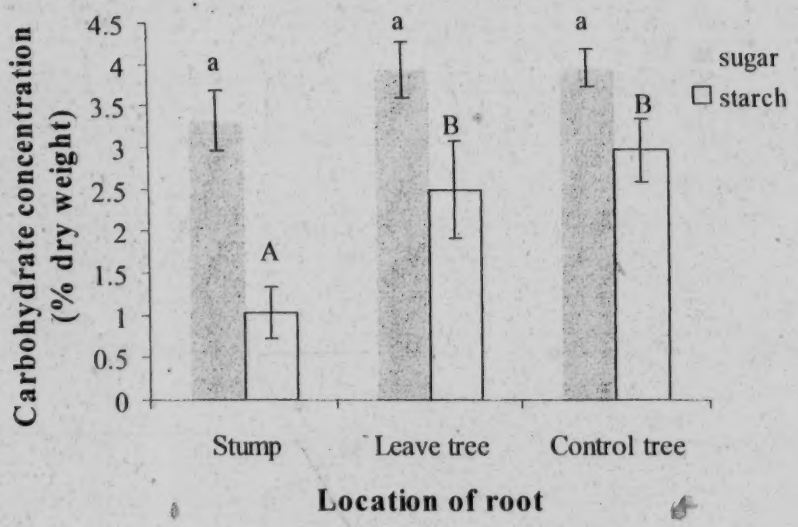

Figure 4-9: Sugar and starch concentrations in living roots of stumps, leave trees and control trees in August 2004. Stumps were from trees cut two growing seasons previously during a manual thinning treatment, the leave trees were grafted to the stumps and the control trees were non-grafted and undisturbed. Bars represent the standard error of the mean. Bars with different letters are significantly different at the 95% confidence level. Data for sugar and starch were analyzed separately.

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Chapter V: Research synthesis and future directions

This dissertation investigated the prevalence and some of the potential impacts of root grafting in lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex Loud.) stands. Specifically, the following research objectives were fulfilled:

- Document the prevalence of root grafting in lodgepole pine stands
- Determine whether stand characteristics affect the frequency of root graft formation
- Establish whether root grafts can transfer sufficient carbohydrate reserves from a source tree to a grafted sink tree to affect the vigour of grafted trees relative to non-grafted trees growing in similar environments
- Investigate the longevity and location of live roots on snags and stumps grafted to living trees
- Determine whether live roots on stumps or snags affect the diameter growth of the living grafted partners

Research synthesis and implications:

The results from Chapter II indicate that overall, 32% of trees in our study areas were grafted to at least one other tree and this value increased to 46% if only trees that were at least 15 years of age were assessed. Indeed, the frequency of root graft formation was primarily affected by stand density, tree diameter and tree age; as these variables increased, the frequency of root grafting also increased. These results along with findings from previous studies (Schultz and Woods 1967, Eis 1972) clearly indicate that root grafts are a common occurrence in many high density coniferous forest stands.

Based on previous work, it is also evident that critical resources like carbohydrates can be transferred among trees via root grafts (Bormann 1961, Bormann 1966), which seems to indicate that intra-specific competition may not be occurring in the strictest sense among grafted trees. In fact, it has been suggested that it may be necessary to re-evaluate some of the accepted concepts of plant competition to incorporate the mutualistic relationships occurring among grafted trees (Kuntz and Riker 1956, Bormann 1962, Eis 1972). Results from Chapter III further strengthen this suggestion as we observed greater root carbohydrate reserves in shaded trees grafted to non-shaded neighbours compared to non-grafted shaded trees (Fig. 3-2). This finding implies that root grafts can transfer enough critical resources to affect the carbon economy of subordinate trees. However, since the shading treatment reduced crown size and stem diameter growth (Table 3-1) in both grafted and non-grafted shaded trees, it appears that the shaded trees were not able to acquire enough resources to sustain both the root system and the more distal above-ground tissues. Indeed, it appears that the size of graft was important in determining how much photosynthate was passed between trees (Fig. 3-4) and in most cases, it is likely that grafts were too small to deliver the carbohydrates needed to completely maintain the grafted shaded neighbours. Nevertheless, because grafted shaded trees had greater root reserves than non-grafted shaded trees, it is possible that subordinate trees grafted to a vigorous neighbour may be able to allocate a greater proportion of photosynthate to stem and crown growth relative to non-grafted trees and this could allow grafted trees to persist longer under shaded conditions. As a result, root grafting could partially explain the slow self-thinning that can occur in some over-stocked forest stands.

Following the death of individual trees (either from natural causes or manual thinning), the base of the stem and the root systems of snags and stumps can be kept alive through the transfer of carbohydrates via root grafts from living neighbouring trees (e.g.: Bormann 1966, Schultz and Woods 1967). Previous studies have assessed the longevity of above-ground portions of stumps and snags following partial cutting or other disturbances and have reported variable results for different species. In general, it appears that species that form callus tissue over the exposed stump or snag surface soon after death seem to be able to survive longer than those species that form only a resin cap on the exposed surface (Newins 1916, Bormann 1961, Lanner 1961) as the callus tissue is much more resistant to fungal and insect attack (Lanner 1961, Miller and Woods 1965). We did not observe any callus tissue formation in the study described in Chapter IV, so it unlikely that live tissue on lodgepole pine snags and stumps would persist for long periods. In fact, the proportion of live roots on lodgepole pine snags and stumps grafted to living trees gradually decreased over time until virtually no live roots remained approximately 15 years after crown death (Fig. 4-3).

Previous studies have attempted to determine whether the living tree is helped or hindered by snag or stump root systems, but the results have been unclear (Walters 1963, Bormann 1966, Eis 1972). Results from Chapter IV, however, suggest that the inheritance of a snag or stump root system does not improve diameter growth rates in living trees. We observed increases in tree ring increment (TRI) in leave trees following the manual thinning of the surrounding grafted partners (Fig. 4-7), but we did not observe a similar response when trees died naturally and became snags. Since the living trees in

both studies would have had access to an increased root absorptive surface area, the living trees should have exhibited improved diameter growth if the inherited root system was beneficial. Instead, it appears that the improved growing conditions experienced by the leave trees following manual thinning were responsible for the improved diameter growth. Further, tracer movement in the xylem tissues of stumps grafted to living trees has been observed to be slow and inefficient (Bormann 1966), which suggests that water uptake by stump roots may be minimal. Conversely, movement of carbohydrates from the living tree to support a relatively large and ineffective root system on the snag or stump could easily overwhelm any positive effects of increased water uptake associated with these roots.

Future directions:

There have been a number of observational studies carried out to assess the prevalence of root grafting in different species (e.g.: Bormann and Graham 1959, Schultz and Woods 1967, Eis 1972, Stone 1974, Chapter II). As a result, the occurrence of root grafting has been well documented. There have also been a number of studies assessing tracer movement among trees that have determined root grafts can transfer critical resources (e.g.: Bormann and Graham 1959, Bormann 1961, Bormann 1966, Schultz and Woods 1967, Stone 1974). However, there has been very little experimental work done with root grafts and it is currently unclear whether root grafts affect tree growth and survival rates independently from other stand characteristics. Considering that root graft frequency is closely linked to stand density (Schultz and Woods 1967, Eis 1972, Chapter II), it is often difficult to separate the effects of density from those of root grafting in

comparisons of growth and/or survival of grafted and non-grafted trees. In fact, the study described in Chapter III has been the only study to date that has attempted to do this. Consequently, it would be valuable to establish a long-term study where seedlings are planted at high densities and the roots of some trees would be fenced off with small barriers to prevent root grafting while the roots of others intermingle freely to encourage root graft formation. Once functional root grafts have formed between trees, a number of questions could be addressed, including the following:

Based on the results from Chapter III, it seems clear that subordinate trees can be partially supported by resources transferred across root grafts from more vigorous partners. However, the shading treatment that was imposed in this study was very severe (<25% light) and was maintained for only one growing season. Also, the trees in each grafted pair were of approximately equal height and crown class. Consequently, the experimental conditions used in this study did not duplicate conditions that would exist under more natural conditions where the subordinate tree would be much smaller than the vigorous neighbour and light conditions would likely not be as limiting. Therefore, it may be useful to record growth and survival rates of both grafted and non-grafted dominant and suppressed trees over a number of years in the hypothetical long-term study area described above so that survival and growth rates could be documented under conditions that would more closely mimic those in a dense natural forest.

In Chapter IV, results indicated that the inheritance of a snag or stump root system was not beneficial to the growth of the grafted living trees. However, it is still

unknown whether the growth of the leave trees was affected by the inheritance of this large grafted root system following manual thinning. Considering that stand density and root grafting frequency are closely linked (e.g.: Chapter II), trees growing in stands that are dense enough to need manual thinning would likely support numerous root grafts with neighbouring trees. Conversely, non-grafted trees would likely be growing a meter or more away from neighbouring trees and would be largely unaffected by manual thinning treatments. Consequently, grafted trees would likely not respond to thinning in the same way as non-grafted trees, simply due to the different growing conditions. However, if a long-term study like the one described above was set up, it would be possible to separate the growth effects of root grafting from those related to stand density. Consequently, it could be determined whether the growth of leave trees grafted to stumps with live tissue was different from the growth of non-grafted trees and whether the size of the grafted root system affected the growth rates of leave trees.

Inter-species root grafting has been documented very rarely, especially in coniferous species (Graham and Bormann 1966). Consequently, it appears that there must be sufficient genetic similarity between roots for root grafts to form. Further, we observed several instances where groups of lodgepole pine trees growing in very close proximity appeared to be connected with numerous root grafts yet one or more trees in the group would not be grafted to any other, even though their roots were highly intertwined and tightly pressed together. This suggests that even within species, a certain degree of genetic compatibility may be necessary for root graft formation. This question

could be addressed by comparing the DNA of trees that are not part of the grafted group with those that are.

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